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#### (54) GLUCOSYLTRANSFERASE ENZYMES FOR PRODUCTION OF GLUCAN POLYMERS

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- (51) Int. Cl. C12N 9/10 (2006.01)C12P 19/18 (2006.01)C08B 37/00 (2006.01)C12P 19/04 (2006.01)
- (52) U.S. Cl.

CPC .......... C12N 9/1051 (2013.01); C08B 37/0009 (2013.01); C12N 9/1048 (2013.01); C12P 19/04 (2013.01); C12P 19/18 (2013.01); C12Y **204/01005** (2013.01)

(58) Field of Classification Search

See application file for complete search history.

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#### **ABSTRACT**

Reaction solutions are disclosed herein comprising water, sucrose and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan. The glucosyltransferase enzyme can synthesize insoluble glucan polymer having at least 50% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100. Further disclosed are methods of using such glucosyltransferase enzymes to produce insoluble poly alpha-1,3-glucan.

#### 22 Claims, No Drawings

# GLUCOSYLTRANSFERASE ENZYMES FOR PRODUCTION OF GLUCAN POLYMERS

This application is a divisional of pending application Ser. No. 14/036,049, filed Sep. 25, 2013, which claims the benefit of U.S. Provisional Application Nos. 61/705,177; 61/705, 178; 61/705,179; 61/705,180 and 61/705,181, each filed Sep. 25, 2012. All of these prior applications are incorporated herein by reference in their entirety.

#### FIELD OF INVENTION

The invention is in the field of enzyme catalysis. Specifically, this invention pertains to producing high molecular weight, insoluble poly alpha-1,3-glucan using a glucosyl
15 hydrolyzed glucan. In a seventh emb

#### BACKGROUND

Driven by a desire to find new structural polysaccharides 20 using enzymatic syntheses or genetic engineering of microorganisms or plant hosts, researchers have discovered polysaccharides that are biodegradable and can be made economically from renewably sourced feedstocks. One such polysaccharide is poly alpha-1,3-glucan, a glucan polymer 25 characterized by having alpha-1,3-glycosidic linkages. This polymer has been isolated by contacting an aqueous solution of sucrose with a glucosyltransferase (gtf) enzyme isolated from *Streptococcus salivarius* (Simpson et al., *Microbiology* 141:1451-1460, 1995). Films prepared from poly alpha-1,3-30 glucan tolerate temperatures up to 150° C. and provide an advantage over polymers obtained from beta-1,4-linked polysaccharides (Ogawa et al., *Fiber Differentiation Methods* 47:353-362, 1980).

U.S. Pat. No. 7,000,000 disclosed the preparation of a <sup>35</sup> polysaccharide fiber using an *S. salivarius* gtfJ enzyme. At least 50% of the hexose units within the polymer of this fiber were linked via alpha-1,3-glycosidic linkages. *S. salivarius* gtfJ enzyme utilizes sucrose as a substrate in a polymerization reaction producing poly alpha-1,3-glucan and fructose as <sup>40</sup> end-products (Simpson et al., 1995). The disclosed polymer formed a liquid crystalline solution when it was dissolved above a critical concentration in a solvent or in a mixture comprising a solvent. Continous, strong, cotton-like fibers were obtained from this solution that could be spun and used <sup>45</sup> in textile applications.

Not all glucosyltransferase enzymes can produce glucan with a molecular weight and percentage of alpha-1,3 glycosidic linkages suitable for use in spinning fibers. For example, most glucosyltransferase enzymes do not produce glucan 50 having at least 50% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100. Therefore, it is desirable to identify glucosyltransferase enzymes that can convert sucrose to glucan polymers having a high percentage of alpha-1,3 glycosidic linkages and high molecular weight.

#### SUMMARY OF INVENTION

In one embodiment, the invention concerns a reaction solution comprising water, sucrose and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan. The glucosyltransferase enzyme comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ 65 ID NO:20, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:34.

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In a second embodiment, the glucosyltransferase enzyme in the reaction solution synthesizes poly alpha-1,3-glucan having at least 50% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100. In a third embodiment, the glucosyltransferase synthesizes poly alpha-1,3-glucan having 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100. In a fourth embodiment, the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 250.

In a fifth embodiment, the reaction solution comprises a primer. In a sixth embodiment, this primer can be dextran or hydrolyzed glucan.

In a seventh embodiment, the invention concerns a method for producing poly alpha-1,3-glucan comprising the step of contacting at least water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan. The glucosyltransferase enzyme comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:20, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:34. The poly alpha-1,3-glucan produced in this method can optionally be isolated.

In an eighth embodiment, the glucosyltransferase enzyme used in the method synthesizes poly alpha-1,3-glucan having at least 50% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100. In a ninth embodiment, the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100. In a tenth embodiment, the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 250.

In an eleventh embodiment, the contacting step of the method further comprises contacting a primer with the water, sucrose, and glucosyltransferase enzyme. In a twelfth embodiment, this primer can be dextran or hydrolyzed glucan

#### BRIEF DESCRIPTION OF THE SEQUENCES

TABLE 1

Summary of Nucleic Acid and Protein SEQ ID	Numbers	
Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.
"0874 gtf", Streptococcus sobrinus. DNA codon- optimized for expression in E. coli. The first 156 amino acids of the protein are deleted compared to GENBANK Identification No. 450874, which discloses "glucosyltransferase-I".	1	2 (1435 aa)
"6855 gtf", Streptococcus salivarius SK126. DNA codon-optimized for expression in E. coli. The first 178 amino acids of the protein are deleted compared to GENBANK Identification No. 228476855, which discloses "glucosyltransferase-SF".	3	4 (1341 aa)
"2379 gtf", Streptococcus salivarius. DNA codon- optimized for expression in E. coli. The first 203 amino acids of the protein are deleted compared to GENBANK Identification No. 662379, which discloses "glucosyltransferase".	5	6 (1247 aa)

# **4** TABLE 1-continued

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Summary of Nucleic Acid and Protein SEQ II	) Numbers	5		Summary of Nucleic Acid and Protein SEQ ID Numbers						
Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.	5	Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.				
"7527" or "gtfJ", Streptococcus salivarius. DNA codon-optimized for expression in E. coli. The first 42 amino acids of the protein are deleted compared to	7	8 (1477 aa)	10	"6907 gtf", Streptococcus salivarius SK126. DNA codon-optimized for expression in E. coli. The first 161 amino acids of the protein are deleted compared	35	36 (1331 aa)				
GENBANK Identification No. 47527, which discloses "glucosyltransferase-I". "1724 gtf", Streptococcus downei. DNA codon-	9	10		to GENBANK Identification No. 228476907, which discloses "glucosyltransferase-SI". "6661 gtf", Streptococcus salivarius SK126. DNA	37	38				
optimized for expression in <i>E. coli</i> . The first 162 amino acids of the protein are deleted compared to GENBANK Identification No. 121724, which discloses "glucosyltransferase-I".		(1436 aa)	15	codon-optimized for expression in <i>E. coli</i> . The first 265 amino acids of the protein are deleted compared to GENBANK Identification No. 228476661, which discloses "glucosyltransferase-SI".		(1305 aa				
"0544 gtf", Streptococcus mutans. DNA codon- optimized for expression in E. coli. The first 164 amino acids of the protein are deleted compared to GENBANK Identification No. 290580544, which	11	12 (1313 aa)		"0339 gtf", Streptococcus gallolyticus ATCC 43143. DNA codon-optimized for expression in E. coli. The first 213 amino acids of the protein are deleted compared to GENBANK Identification No.	39	40 (1310 aa				
discloses "glucosyltransferase-I". "5926 gtf", Streptococcus dentirousetti. DNA codon- optimized for expression in E. coli. The first 144 amino acids of the protein are deleted compared to GENBANK Identification No. 167735926, which	13	14 (1323 aa)	20	334280339, which discloses "glucosyltransferase". "0088 gtf", <i>Streptococcus mutans</i> . DNA codonoptimized for expression in <i>E. coli</i> . The first 189 amino acids of the protein are deleted compared to GENBANK Identification No. 3130088, which	41	42 (1267 aa				
discloses "glucosyltransferase-I". "4297 gt", Streptococcus oralis. DNA codon- optimized for expression in E. coli. The first 228 amino acids of the protein are deleted compared to GENBANK Identification No. 7684297, which	15	16 (1348 aa)	25	discloses "glucosyltransferase-SI".  "9358 gtf", <i>Streptococcus mutans</i> UA159. DNA codon-optimized for expression in <i>E. coli</i> . The first 176 amino acids of the protein are deleted compared to GENBANK Identification No. 24379358, which	43	44 (1287 aa				
discloses "glucosyltransferase". "5618 gtf", Streptococcus sanguinis. DNA codon- optimized for expression in E. coli. The first 223 amino acids of the protein are deleted compared to	17	18 (1348 aa)	30	discloses "glucosyltransferase-S". "8242 gtf", <i>Streptococcus gallolyticus</i> ATCC BAA-2069. DNA codon-optimized for expression in <i>E. coli</i> . The first 191 amino acids of the protein are deleted	45	46 (1355 aa				
GENBANK Identification No. 328945618, which discloses "glucosyltransferase-S". "2765 gff", unknown <i>Streptococcus</i> sp. C150. DNA codon-optimized for expression in <i>E. coli</i> . The first 193 amino acids of the protein are deteed compared to GENBANK Identification. No. 20227765, which	19	20 (1340 aa)		compared to GENBANK Identification No. 325978242, which discloses "glucosyltransferase-I". "3442 gtf", <i>Streptococcus sanguinis</i> SK405. DNA codon-optimized for expression in <i>E. coli</i> . The first 228 amino acids of the protein are deleted compared to GENBANK Identification No. 224003443, which	47	48 (1348 aa				
to GENBANK Identification No. 322372765, which discloses "glucosyltransferase-S". "4700 gtf", Leuconostoc mesenteroides. DNA codon-optimized for expression in E. coli. The first 36 amino acids of the protein are deleted compared to GENBANK Identification No. 21654700, which	21	22 (1492 aa)	35	to GENBANK Identification No. 324993442, which discloses a " signal domain protein". "7528 gtf", <i>Streptococcus salivarius</i> . DNA codon-optimized for expression in <i>E. coli</i> . The first 173 amino acids of the protein are deleted compared to GENBANK Identification No. 47528, which discloses	49	50 (1427 aa				
discloses "dextransucrase DsrD".  "1366 gtf", Streptococcus criceti. DNA codonoptimized for expression in E. coli. The first 139 amino acids of the protein are deleted compared to GENBANK Identification No. 146741366, which	23	24 (1323 aa)	40	"glucosyltransferase S". "3279 gtf", <i>Streptococcus</i> sp. C150. DNA codon- optimized for expression in <i>E. coli</i> . The first 178 amino acids of the protein are deleted compared to GENBANK Identification No. 322373279, which	51	52 (1393 aa				
discloses "glucosyltransferase".  "0427 gtf", Streptococcus sobrinus. DNA codon- optimized for expression in E. coli. The first 156 amino acids of the protein are deleted compared to GENBANK Identification No. 940427, which	25	26 (1435 aa)	45	discloses "glucosyltransferase S". "6491 gtf", Leuconostoc citreum KM20. DNA codon- optimized for expression in E. coli. The first 244 amino acids of the protein are deleted compared to GENBANK Identification No. 170016491, which discloses "glucosyltransferase".	53	54 (1262 aa				
discloses "GTF-I".  "2919 gtf", Streptococcus salivarius PS4. DNA codon-optimized for expression in E. coli. The first 92 amino acids of the protein are deleted compared to GENBANK Identification No. 383282919, which	27	28 (1340 aa)	50	"6889 gtf", Streptococcus salivarius SK126. DNA codon-optimized for expression in E. coli. The first 173 amino acids of the protein are deleted compared to GENBANK Identification No. 228476889, which discloses "glucosyltransferase-I".	55	56 (1427 aa				
discloses "putative glucosyltransferase".  "2678 gtf", Streptococcus salivarius K12. DNA codonoptimized for expression in E. coli. The first 188 amino acids of the protein are deleted compared to GENBANK Identification No. 400182678, which	29	30 (1341 aa)	55	"4154 gtf", <i>Lactobacillus reuteri</i> . DNA codon- optimized for expression in <i>E. coli</i> . The first 38 amino acids of the protein are deleted compared to GENBANK Identification No. 51574154, which discloses "glucansucrase".	57	58 (1735 aa				
"2381 gtf", Streptococcus salivarius. DNA codon- optimized for expression in E. coli. The first 273 amino acids of the protein are deleted compared to GENBANK Identification No. 662381, which	31	32 (1305 aa)	60	"3298 gtf", Streptococcus sp. C150. The first 209 amino acids of the protein are deleted compared to GENBANK Identification No. 322373298, which discloses "glucosyltransferase-S". "Wild type gtff", Streptococcus salivarius.		59 (1242 aa				
discloses "glucosyltransferase".  "3929 gtf", Streptococcus salivarius JIM8777. DNA codon-optimized for expression in E. coli. The first 178 amino acids of the protein are deleted compared to GENBANK Identification No. 387783929, which	33	34 (1341 aa)		Wild type gits , Sureptococus survivus.  GENBANK Identification No. 47527.  Wild type gtf corresponding to 2678 gtf, Streptococcus salivarius K12. GENBANK Identification No. 400182678, which discloses "dextransucrase-S".  Wild type gtf corresponding to 6855 gtf, Streptococcus		(1518 aa 61 (1528 aa				
to GENBANK Identification No. 387/85929, Which discloses "glucosyltransferase-S precursor (GTF-S) (Dextransucrase) (Sucrose 6-glucosyltransferase)".			65	what type git corresponding to 6855 gtt, <i>streptococcus</i> salivarius SK126. GENBANK Identification No. 228476855, which discloses "glucosyltransferase-SI".		(1518 aa				

Summary of Nucleic Acid and Protein SEQ ID Numbers											
Description	Nucleic acid SEQ ID NO.	Protein SEQ ID NO.									
Wild type gtf corresponding to 2919 gtf, Streptococcus salivarius PS4. GENBANK Identification No. 383282919, which discloses "putative glucosyltransferase".		63 (1431 aa)									
which discloses "glucosyltransferase-S".		64 (1532 aa)									

#### DETAILED DESCRIPTION OF THE INVENTION

The disclosures of all cited patent and non-patent literature are incorporated herein by reference in their entirety.

As used herein, the term "invention" or "disclosed invention" is not meant to be limiting, but applies generally to any of the inventions defined in the claims or described herein. These terms are used interchangeably herein.

The terms "poly alpha-1,3-glucan", "alpha-1,3-glucan <sub>25</sub> polymer" and "glucan polymer" are used interchangeably herein. Poly alpha-1,3-glucan is a polymer comprising glucose monomeric units linked together by glycosidic linkages, wherein at least about 50% of the glycosidic linkages are alpha-1,3-glycosidic linkages. Poly alpha-1,3-glucan is a <sub>30</sub> type of polysaccharide. The structure of poly alpha-1,3-glucan can be illustrated as follows:

The terms "glycosidic linkage" and "glycosidic bond" are used interchangeably herein and refer to the type of covalent bond that joins a carbohydrate (sugar) molecule to another group such as another carbohydrate. The term "alpha-1,3-45 glycosidic linkage" as used herein refers to the type of covalent bond that joins alpha-D-glucose molecules to each other through carbons 1 and 3 on adjacent alpha-D-glucose rings. This linkage is illustrated in the poly alpha-1,3-glucan structure provided above. Herein, "alpha-D-glucose" will be 50 referred to as "glucose".

The term "sucrose" herein refers to a non-reducing disaccharide composed of an alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1,2-glycosidic bond. Sucrose is known commonly as table sugar.

The "molecular weight" of the poly alpha-1,3-glucan herein can be represented as number-average molecular weight  $(M_n)$  or as weight-average molecular weight  $(M_w)$ . Alternatively, molecular weight can be represented as Daltons, grams/mole, DPw (weight average degree of polymerization), or DPn (number average degree of polymerization). Various means are known in the art for calculating these molecular weight measurements such as with high-pressure liquid chromatography (HPLC), size exclusion chromatography (SEC), or gel permeation chromatography (GPC).

The terms "glucosyltransferase enzyme", "gtf enzyme", "gtf enzyme catalyst", "gtf", and "glucansucrase" are used

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interchangeably herein. The activity of a gtf enzyme herein catalyzes the reaction of the substrate sucrose to make the products poly alpha-1,3-glucan and fructose. Other products (byproducts) of a gtf reaction can include glucose (where glucose is hydrolyzed from the glucosyl-gtf enzyme intermediate complex), various soluble oligosaccharides (DP2-DP7), and leucrose (where glucose of the glucosyl-gtf enzyme intermediate complex is linked to fructose). Leucrose is a disaccharide composed of glucose and fructose linked by an alpha-10 1,5 linkage. Wild type forms of glucosyltransferase enzymes generally contain (in the N-terminal to C-terminal direction) a signal peptide, a variable domain, a catalytic domain, and a glucan-binding domain. A gtf herein is classified under the glycoside hydrolase family 70 (GH70) according to the 15 CAZy (Carbohydrate-Active EnZymes) database (Cantarel et al., Nucleic Acids Res. 37:D233-238, 2009).

The terms "reaction" and "enzymatic reaction" are used interchangeably herein and refer to a reaction that is performed by a glucosyltransferase enzyme. A "reaction solution" as used herein generally refers to a solution comprising at least one active glucosyltransferase enzyme in a solution comprising sucrose and water, and optionally other components. It is in the reaction solution where the step of contacting water, sucrose and a glucosyltransferase enzyme is performed. The term "under suitable reaction conditions" as used herein, refers to reaction conditions that support conversion of sucrose to poly alpha-1,3-glucan via glucosyltransferase enzyme activity. The reaction herein is not naturally occurring.

The terms "percent by volume", "volume percent", "vol %" and "v/v %" are used interchangeably herein. The percent by volume of a solute in a solution can be determined using the formula: [(volume of solute)/(volume of solution)]× 100%

The terms "percent by weight", "weight percentage (wt %)" and "weight-weight percentage (% w/w)" are used interchangeably herein. Percent by weight refers to the percentage of a material on a mass basis as it is comprised in a composition, mixture, or solution.

The terms "increased", "enhanced" and "improved" are used interchangeably herein. These terms refer to a greater quantity or activity such as a quantity or activity slightly greater than the original quantity or activity, or a quantity or activity in large excess compared to the original quantity or activity, and including all quantities or activities in between. Alternatively, these terms may refer to, for example, a quantity or activity that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% more than the quantity or activity for which the increased quantity or activity is being compared.

The terms "polynucleotide", "polynucleotide sequence", and "nucleic acid sequence" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of DNA or RNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof.

The term "gene" as used herein refers to a polynucleotide sequence that expresses a protein, and which may refer to the coding region alone or may include regulatory sequences upstream and/or downstream to the coding region (e.g., 5' untranslated regions upstream of the transcription start site of the coding region). A gene that is "native" or "endogenous" refers to a gene as found in nature with its own regulatory sequences; this gene is located in its natural location in the genome of an organism. "Chimeric gene" refers to any gene

that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. A "foreign" or "heterologous" gene refers to a gene that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, native 5 genes introduced into a new location within the native host, or chimeric genes. The polynucleotide sequences in certain embodiments disclosed herein are heterologous. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. A "codon-optimized gene" is a 10 gene having its frequency of codon usage designed to mimic the frequency of preferred codon usage of the host cell.

A native amino acid sequence or polynucleotide sequence is naturally occurring, whereas a non-native amino acid sequence or polynucleotide sequence does not occur in 15 nature.

"Coding sequence" as used herein refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" as used herein refer to nucleotide sequences located upstream of the coding sequence's tran-20 scription start site, 5' untranslated regions and 3' non-coding regions, and which may influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, polyadenylation recognition sequences, RNA processing sites, effector binding sites, stem-loop structures and other elements involved in regulation of gene expression.

The term "recombinant" as used herein refers to an artificial combination of two otherwise separated segments of 30 sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques. The terms "recombinant", "transgenic", "transformed", "engineered" or "modified for exogenous gene expression" are used interchangeably herein.

The term "transformation" as used in certain embodiments refers to the transfer of a nucleic acid molecule into a host organism. The nucleic acid molecule may be a plasmid that replicates autonomously, or it may integrate into the genome formed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms or "transformants".

The term "recombinant" or "heterologous" refers to an artificial combination of two otherwise separate segments of 45 sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

The terms "sequence identity" or "identity" as used herein with respect to polynucleotide or polypeptide sequences refer 50 to the nucleic acid bases or amino acid residues in two sequences that are the same when aligned for maximum correspondence over a specified comparison window. Thus, "percentage of sequence identity" or "percent identity" refers to the value determined by comparing two optimally aligned 55 sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two 60 sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the 65 window of comparison and multiplying the results by 100 to yield the percentage of sequence identity.

The Basic Local Alignment Search Tool (BLAST) algorithm, which is available online at the National Center for Biotechnology Information (NCBI) website, may be used, for example, to measure percent identity between or among two or more of the polynucleotide sequences (BLASTN algorithm) or polypeptide sequences (BLASTP algorithm) disclosed herein. Alternatively, percent identity between sequences may be performed using a Clustal algorithm (e.g., ClustalW or ClustalV). For multiple alignments using a Clustal method of alignment, the default values may correspond to GAP PENALTY=10 and GAP LENGTH PEN-ALTY=10. Default parameters for pairwise alignments and calculation of percent identity of protein sequences using a Clustal method may be KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids, these parameters may be KTUPLE=2, GAP PEN-ALTY=5, WINDOW=4 and DIAGONALS SAVED=4. Alternatively still, percent identity between sequences may be performed using an EMBOSS algorithm (e.g., needle) with parameters such as GAP OPEN=10, GAP EXTEND=0.5, END GAP PENALTY=false, END GAP OPEN=10, END GAP EXTEND=0.5 using a BLOSUM matrix (e.g., BLOSUM62).

Various polypeptide amino acid sequences and polynucleenhancers, silencers, 5' untranslated leader sequence, introns, 25 otide sequences are disclosed herein as features of certain embodiments of the disclosed invention. Variants of these sequences that are at least about 70-85%, 85-90%, or 90%-95% identical to the sequences disclosed herein can be used. Alternatively, a variant amino acid sequence or polynucleotide sequence can have at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with a sequence disclosed herein. The variant amino acid sequence or polynucle-35 otide sequence has the same function/activity of the disclosed sequence, or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the function/activity of the disclosed sequence.

The term "isolated" as used in certain embodiments refers of the host organism. Host organisms containing the trans- 40 to any cellular component that has been completely or partially purified from its native source (e.g., an isolated polynucleotide or polypeptide molecule). In some instances, an isolated polynucleotide or polypeptide molecule is part of a greater composition, buffer system or reagent mix. For example, the isolated polynucleotide or polypeptide molecule can be comprised within a cell or organism in a heterologous manner. Another example is an isolated glucosyltransferase enzyme.

Embodiments of the disclosed invention concern a reaction solution comprising water, sucrose and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan. The glucosyltransferase enzyme comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:20, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:34. Significantly, these glucosyltransferase enzymes can synthesize poly alpha-1,3-glucan having at least 50% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100. Such glucan is suitable for use in spinning fibers and in other industrial applications.

The molecular weight of the poly alpha-1,3-glucan produced by the glucosyltransferase enzymes herein can be measured as  $DP_n$  (number average degree of polymerization). Alternatively, the molecular weight of the poly alpha-1,3glucan can be measured in terms of Daltons, grams/mole, or as DP<sub>w</sub> (weight average degree of polymerization). The poly

alpha-1,3-glucan in certain embodiments of the invention can have a molecular weight in  $DP_n$  or  $DP_w$  of at least about 100. The molecular weight of the poly alpha-1,3-glucan can alternatively be at least about 250  $DP_n$  or  $DP_w$ . Alternatively still, the DP<sub>n</sub> or DP<sub>w</sub> of the poly alpha-1,3-glucan can be at least about 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 (or any integer between 100 and 1000).

The molecular weight of the poly alpha-1,3-glucan herein can be measured using any of several means known in the art. For example, glucan polymer molecular weight can be measured using high-pressure liquid chromatography (HPLC), size exclusion chromatography (SEC), or gel permeation chromatography (GPC).

The poly alpha-1,3-glucan herein is preferably linear/unbranched. The percentage of glycosidic linkages between the glucose monomer units of the poly alpha-1,3-glucan that are alpha-1,3 is at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In such embodiments, 20 accordingly, the poly alpha-1,3-glucan has less than about 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1%, or 0% of glycosidic linkages that are not alpha-1,3.

It is understood that the higher the percentage of alpha-1, 3-glycosidic linkages present in the poly alpha-1,3-glucan, <sup>25</sup> the greater the probability that the poly alpha-1,3-glucan is linear, since there are lower occurrences of certain glycosidic linkages forming branch points in the polymer. In certain embodiments, the poly alpha-1,3-glucan has no branch points or less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% branch points as a percent of the glycosidic linkages in the polymer. Examples of branch points include alpha-1,6 branch points, such as those that are present in mutan poly-

The glycosidic linkage profile of the poly alpha-1,3-glucan can be determined using any method known in the art. For example, the linkage profile can be determined using methods that use nuclear magnetic resonance (NMR) spectroscopy can be used are disclosed in Food Carbohydrates: Chemistry. Physical Properties, and Applications (S. W. Cui, Ed., Chapter 3, S. W. Cui, Structural Analysis of Polysaccharides, Taylor & Francis Group LLC, Boca Raton, Fla., 2005), which is incorporated herein by reference.

The poly alpha-1,3-glucan herein may be characterized by any combination of the aforementioned percentages of alpha-1,3 linkages and molecular weights. For example, the poly alpha-1,3-glucan produced in a reaction solution herein can have at least 50% alpha-1,3 glycosidic linkages and a  $DP_n$  or 50 DP<sub>w</sub> of at least 100. As another example, the poly alpha-1,3glucan can have 100% alpha-1,3 glycosidic linkages and a DP, or DP, of at least 100. The poly alpha-1,3-glucan in still another example can have 100% alpha-1,3 glycosidic linkages and a  $DP_n$  or  $DP_w$  of at least 250.

The glucosyltransferase enzyme in certain embodiments of the invention may be derived from a *Streptococcus* species, Leuconostoc species or Lactobacillus species, for example. Examples of *Streptococcus* species from which the glucosyltransferase may be derived include S. salivarius, S. sobrinus, S. dentirousetti, S. downei, S. mutans, S. oralis, S. gallolyticus and S. sanguinis. Examples of Leuconostoc species from which the glucosyltransferase may be derived include L. mesenteroides, L. amelibiosum, L. argentinum, L. carnosum, L. citreum, L. cremoris, L. dextranicum and L. fructosum. 65 Examples of *Lactobacillus* species from which the glucosyltransferase may be derived include L. acidophilus, L. del10

brueckii, L. helveticus, L. salivarius, L. casei, L. curvatus, L. plantarum, L. sakei, L. brevis, L. buchneri, L. fermentum and L. reuteri.

The glucosyltransferase enzyme herein can comprise, or consist of, an amino acid sequence that is at least 90% identical to the amino acid sequence provided in SEO ID NO:4. SEO ID NO:10, SEO ID NO:12, SEO ID NO:14, SEO ID NO:20, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:34, wherein the glucosyltransferase enzyme has activity. Alternatively, the glucosyltransferase enzyme can comprise, or consist of, an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:20, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:34, wherein the glucosyltransferase enzyme has activity.

All the amino acid residues disclosed herein at each amino acid position of the glucosyltransferase enzyme sequences are examples. Given that certain amino acids share similar structural and/or charge features with each other (i.e., conserved), the amino acid at each position in the glucosyltransferase enzyme sequences can be as provided in the disclosed sequences or substituted with a conserved amino acid residue ("conservative amino acid substitution") as follows:

- 1. The following small aliphatic, nonpolar or slightly polar residues can substitute for each other: Ala (A), Ser (S), Thr (T), Pro (P), Gly (G);
- 2. The following polar, negatively charged residues and their amides can substitute for each other: Asp (D), Asn (N), Glu (E), Gln (Q);
- 3. The following polar, positively charged residues can substitute for each other: H is (H), Arg (R), Lys (K);
- 4. The following aliphatic, nonpolar residues can substitute for each other: Ala (A), Leu (L), Ile (I), Val (V), Cys (C), Met (M): and
- 5. The following large aromatic residues can substitute for each other: Phe (F), Tyr (Y), Trp (W).

Examples of glucosyltransferase enzymes may be any of (e.g., 13C NMR or 1H NMR). These and other methods that 40 the amino acid sequences disclosed herein and that further include 1-300 (or any integer there between) residues on the N-terminus and/or C-terminus. Such additional residues may be from a corresponding wild type sequence from which the glucosyltransferase enzyme is derived, or may be another sequence such as an epitope tag (at either N- or C-terminus) or a heterologous signal peptide (at N-terminus), for example. Thus, examples of glucosyltransferase enzymes include SEQ ID NOs:61, 62, 63 and 64, which represent the wild type sequences from which SEQ ID NOs:30, 4, 28 and 20 are derived, respectively.

The glucosyltransferase enzyme can be encoded by the polynucleotide sequence provided in SEQ ID NO:3, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:33, for example. Alternatively, the glucosyltransferase enzyme can be encoded by a polynucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:3, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:33.

The glucosyltransferase enzyme in certain embodiments synthesizes poly alpha-1,3-glucan in which at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% (or any integer between 50% and 100%) of the constituent glycosidic linkages are alpha-1,3 linkages. In such embodiments, accordingly, the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan in which there is less than

about 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% of glycosidic linkages that are not alpha-1,3.

In other aspects, the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan with no branch points or less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% branch points as a percent of the glycosidic linkages in the polymer. Examples of branch points include alpha-1,6 branch points, such as those that are present in mutan polymer.

The glucosyltransferase enzyme can synthesize poly alpha-1,3-glucan having a molecular weight in  $DP_n$  or  $DP_w$  of 10 at least about 100. Alternatively, the glucosyltransferase enzyme can synthesize poly alpha-1,3-glucan having a molecular weight in  $DP_n$  or  $DP_w$  of at least about 400. Alternatively still, the glucosyltransferase enzyme can synthesize poly alpha-1,3-glucan having a molecular weight in  $DP_n$  or 15  $DP_w$  of at least about 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 (or any integer between 100 and 1000).

One or more different glucosyltransferase enzymes may be used in the disclosed invention. The glucosyltransferase 20 enzyme preferably does not have, or has very little (less than 1%), dextransucrase, reuteransucrase, or alternansucrase activity. The glucosyltransferase in certain embodiments does not comprise amino acid residues 2-1477 of SEQ ID NO:8 or amino acid residues 138-1477 of SEQ ID NO:8, 25 which are derived from the glucosyltransferase identified in GENBANK under GI number 47527 (SEQ ID NO:60).

The glucosyltransferase enzyme herein can be primer-independent or primer-dependent. Primer-independent glucosyltransferase enzymes do not require the presence of a 30 primer to perform glucan synthesis. A primer-dependent glucosyltransferase enzyme requires the presence of an initiating molecule in the reaction solution to act as a primer for the enzyme during glucan polymer synthesis. The term "primer" as used herein refers to any molecule that can act as the 35 initiator for a glucosyltransferase enzyme. Oligosaccharides and polysaccharides can serve a primers herein, for example. Primers that can be used in certain embodiments include dextran and other carbohydrate-based primers, such as hydrolyzed glucan, for example. Hydrolyzed glucan can be pre- 40 pared by acid hydrolysis of a glucan such as poly alphaglucan. International Appl. Publ. No. WO2013/036918, which is incorporated herein by reference, discloses such preparation of hydrolyzed glucan using poly alpha-1,3-glucan as the starting material. Dextran for use as a primer herein 45 can be dextran T10 (i.e., dextran having a molecular weight of 10 kD). Alternatively, the dextran can have a molecular weight of about 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, or 25 kD, for example.

The glucosyltransferase enzyme used herein may be produced by any means known in the art (e.g., U.S. Pat. No. 7,000,000, which is incorporated herein by reference). For example, the glucosyltransferase enzyme may be produced recombinantly in any bacterial (e.g., *E. coli* such as TOP10, *Bacillus* sp.) or eukaryotic (e.g., yeasts such as *Pichia* sp. and *Saccharomyces* sp.) heterologous gene expression system. Any of the above-listed nucleic acid sequences can be used for this purpose, for example.

The glucosyltransferase enzyme used herein may be purified and/or isolated prior to its use, or may be used in the form of a cell lysate, for example. A cell lysate or extract may be prepared from a bacteria (e.g., *E. coli*) used to heterologously express the enzyme. For example, the bacteria may be subjected to disruption using a French pressure cell (French press). The glucosyltransferase enzyme is soluble in these 65 type of preparations. The lysate or extract may be used at about 0.15-0.3% (v/v) in a reaction solution for producing

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poly alpha-1,3-glucan from sucrose. In certain embodiments, a bacterial cell lysate is first cleared of insoluble material by means such as centrifugation or filtration.

In certain embodiments, the heterologous gene expression system may be one that is designed for protein secretion. The glucosyltransferase enzyme comprises a signal peptide (signal sequence) in such embodiments. The signal peptide may be either its native signal peptide or a heterologous signal peptide.

The activity of the glucosyltransferase enzyme can be determined using any method known in the art. For example, glucosyltransferase enzyme activity can be determined by measuring the production of reducing sugars (fructose and glucose) in a reaction solution containing sucrose (50 g/L), dextran T10 (1 mg/mL) and potassium phosphate buffer (pH 6.5, 50 mM), where the solution is held at 22-25° C. for 24-30 hours. The reducing sugars can be measured by adding 0.01 mL of the reaction solution to a mixture containing 1 N NaOH and 0.1% triphenyltetrazolium chloride and then monitoring the increase in absorbance at  $\mathrm{OD}_{480nm}$  for five minutes.

The temperature of the reaction solution herein can be controlled, if desired. In certain embodiments, the solution has a temperature between about 5° C. to about 50° C. The temperature of the solution in certain other embodiments is between about 20° C. to about 40° C. Alternatively, the temperature of the solution may be about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40° C.

The temperature of the reaction solution may be maintained using various means known in the art. For example, the temperature of reaction solution can be maintained by placing the vessel containing the reaction solution in an air or water bath incubator set at the desired temperature.

The initial concentration of the sucrose in the solution can be about 20 g/L to about 400 g/L, for example. Alternatively, the initial concentration of the sucrose can be about 75 g/L to about 175 g/L, or from about 50 g/L to about 150 g/L. Alternatively still, the initial concentration of the sucrose can be about 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, or  $160 \, \text{g/L}$  (or any integer between 40 and  $160 \, \text{g/L}$ ), for example. The "initial concentration of sucrose" refers to the sucrose concentration in the solution just after all the reaction solution components have been added (water, sucrose, gtf enzyme).

Sucrose used in the reaction solution can be highly pure 99.5%) or be of any other purity or grade. For example, the sucrose can have a purity of at least 99.0%, or be reagent grade sucrose. The sucrose may be derived from any renewable sugar source such as sugar cane, sugar beets, cassava, sweet sorghum, or corn. The sucrose can be provided in any form such as crystalline form or non-crystalline form (e.g., syrup or cane juice).

The pH of the reaction solution herein can be between about 4.0 to about 8.0. Alternatively, the pH can be about 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0. In certain embodiments, the pH of a solution containing water and sucrose may be set before adding the glucosyltransferase enzyme. The pH of the reaction solution can be adjusted or controlled by the addition or incorporation of a suitable buffer, including but not limited to: phosphate, tris, citrate, or a combination thereof. The concentration of the buffer can be from 0 mM to about 100 mM, or about 10, 20, or 50 mM, for example. A suitable amount of DTT (dithiothreitol, e.g., about 1.0 mM) can optionally be added to the reaction solution.

The disclosed invention also concerns a method for producing poly alpha-1,3-glucan comprising the step of contacting at least water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan. The glucosyltransferase enzyme can comprise an amino acid sequence that is at

least 90% identical to the amino acid sequence of SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:20, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:34. The poly alpha-1,3-glucan produced in this method can optionally be isolated.

Water, sucrose, and a glucosyltransferase enzyme as described herein are contacted in a reaction solution. Thus, the method can comprise providing a reaction solution comprising water, sucrose and a glucosyltransferase enzyme as described herein. It will be understood that, as the glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan, the reaction solution becomes a reaction mixture given that insoluble poly alpha-1,3-glucan falls out of solution as indicated by clouding of the reaction. The contacting step of the disclosed method can be performed in any number of ways. For example, the desired amount of sucrose can first be dissolved in water (optionally, other components may also be added at this stage of preparation, such as buffer components), followed by the addition of the glucosyltransferase enzyme. The solution may be kept still, or agitated via stirring or orbital shaking, for example. The reaction can be, and 20 typically is, cell-free.

The glucosyltransferase enzyme can optionally be added to water or an aqueous solution (e.g., sucrose in water) that does not contain salt or buffer when initially preparing the reaction solution. The pH of such a preparation can then be modified as desired, such as to pH 5-6 for example. The reaction can be carried out to completion without any added buffer, if desired.

Completion of the reaction in certain embodiments can be determined visually (no more accumulation of precipitated poly alpha-1,3-glucan) and/or by measuring the amount of sucrose left in the solution (residual sucrose), where a percent sucrose consumption of over about 90% can indicate reaction completion. Typically, a reaction of the disclosed process will take about 12, 24, 36, 48, 60, 72, 84, or 96 hours to complete, depending on certain parameters such as the amount of sucrose and glucosyltransferase enzyme used in the reaction.

The percent sucrose consumption of a reaction in certain embodiments of the disclosed process is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. Alternatively, the percent sucrose consumption may be >90% or >95%.

The yield of the poly alpha-1,3-glucan produced in the disclosed invention can be at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20%, based on the weight of the sucrose used in the reaction solution.

The poly alpha-1,3-glucan produced in the disclosed method may optionally be isolated. For example, insoluble poly alpha-1,3-glucan may be separated by centrifugation or filtration. In doing so, the poly alpha-1,3-glucan is separated from the rest of the reaction solution, which may comprise 50 water, fructose and certain byproducts (e.g., leucrose, soluble oligosaccharides DP2-DP7). This solution may also comprise residual sucrose and glucose monomer.

Poly alpha-1,3 glucan is a potentially low cost polymer which can be enzymatically produced from renewable 55 resources containing sucrose using glucosyltransferase enzymes. It has been shown that this polymer can form ordered liquid crystalline solutions when the polymer is dissolved in a solvent under certain conditions (U.S. Pat. No. 7,000,000). Such solutions can be spun into continuous, high 60 strength, cotton-like fibers. The poly alpha-1,3-glucan produced using the disclosed invention has comparable utilities.

#### **EXAMPLES**

The disclosed invention is further defined in the following Examples. It should be understood that these Examples,

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while indicating certain preferred aspects of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.

#### Abbreviations

The meanings of some of the abbreviations used herein are as follows: "g" means gram(s), "h" means hour(s), "mL" means milliliter(s), "psi" means pound(s) per square inch, "wt %" means weight percentage, "µm" means micrometer(s), "o C." means degrees Celsius, "mg" means milligram(s), "mm" means millimeter(s), "µL" means microliter(s), "mmol" means millimole(s), "min" means minute(s), "mol %" means mole percent, "M" means molar, "rpm" means revolutions per minute, "MPa" means megaPascals. General Methods

Preparation of Crude Extracts of Glucosyltransferase (Gtf) Enzymes

Gtf enzymes were prepared as follows. E. coli TOP10® cells (Invitrogen, Carlsbad Calif.) were transformed with a pJexpress404®-based construct containing a particular gtfencoding DNA sequence. Each sequence was codon-optimized to express the gtf enzyme in E. coli. Individual E. coli strains expressing a particular gtf enzyme were grown in LB (Luria broth) medium (Becton, Dickinson and Company, Franklin Lakes, N.J.) with ampicillin (100  $\mu g/mL$ ) at 37° C. with shaking to OD<sub>600</sub>=0.4-0.5, at which time IPTG (isopropyl beta-D-1-thiogalactopyranoside, Cat. No. 16758, Sigma-Aldrich, St. Louis, Mo.) was added to a final concentration of 0.5 mM. The cultures were incubated for 2-4 hours at  $37^{\circ}$  C. following IPTG induction. Cells were harvested by centrifugation at 5,000×g for 15 minutes and resuspended (20% w/v) in 50 mM phosphate buffer pH 7.0 supplemented with dithiothreitol (DTT, 1.0 mM). Resuspended cells were passed through a French Pressure Cell (SLM Instruments, Rochester, N.Y.) twice to ensure >95% cell lysis. Lysed cells were centrifuged for 30 minutes at 12,000×g at 4° C. The resulting supernatant was analyzed by the BCA (bicinchoninic acid) protein assay (Sigma-Aldrich) and SDS-PAGE to confirm expression of the gtf enzyme, and the supernatant was stored at -20° C.

#### Determination of Gtf Enzymatic Activity

Gtf enzyme activity was confirmed by measuring the production of reducing sugars (fructose and glucose) in a gtf reaction solution. A reaction solution was prepared by adding a gtf extract (prepared as above) to a mixture containing sucrose (50 or 150 g/L), potassium phosphate buffer (pH 6.5, 50 mM), and optionally dextran (1 mg/mL, dextran T10, Cat. No. D9260, Sigma-Aldrich); the gtf extract was added to 2.5%-5% by volume. The reaction solution was then incubated at 22-25° C. for 24-30 hours, after which it was centrifuged. Supernatant (0.01 mL) was added to a mixture containing 1 N NaOH and 0.1% triphenyltetrazolium chloride (Sigma-Aldrich). The mixture was incubated for five minutes after which its  $\mathrm{OD}_{480nm}$  was determined using an ULTRO-SPEC spectrophotometer (Pharmacia LKB, New York, N.Y.) to gauge the presence of the reducing sugars fructose and glucose.

#### Determination of Glycosidic Linkages

Glycosidic linkages in the glucan product synthesized by a gtf enzyme were determined by <sup>13</sup>C NMR (nuclear magnetic resonance). Dry glucan polymer (25-30 mg) was dissolved in 1 mL of deuterated dimethyl sulfoxide (DMSO) containing 3% by weight of LiCl with stirring at 50° C. Using a glass pipet, 0.8 mL of the solution was transferred into a 5-mm

NMR tube. A quantitative <sup>13</sup>C NMR spectrum was acquired using a Bruker Avance 500-MHz NMR spectrometer (Billerica, Mass.) equipped with a CPDUL cryoprobe at a spectral frequency of 125.76 MHz, using a spectral window of 26041.7 Hz. An inverse gated decoupling pulse sequence using waltz decoupling was used with an acquisition time of 0.629 second, an inter-pulse delay of 5 seconds, and 6000 pulses. The time domain data was transformed using an exponential multiplication of 2.0 Hz.

Determination of Number Average Degree of Polymerization <sup>10</sup> (DP...)

The DP, of a glucan product synthesized by a gtf enzyme was determined by size-exclusion chromatography (SEC). Dry glucan polymer was dissolved at 5 mg/mL in N,N-dimethyl-acetamide (DMAc) and 5% LiCl with overnight shaking at 100° C. The SEC system used was an Alliance™ 2695 separation module from Waters Corporation (Milford, Mass.) coupled with three on-line detectors: a differential refractometer 2410 from Waters, a multiangle light scattering photometer Heleos<sup>TM</sup> 8+ from Wyatt Technologies (Santa Bar- <sup>20</sup> bara, Calif.), and a differential capillary viscometer ViscoStar<sup>TM</sup> from Wyatt. The columns used for SEC were four styrene-divinyl benzene columns from Shodex (Japan) and two linear KD-806M, KD-802 and KD-801 columns to improve resolution at the low molecular weight region of a  $^{25}$ polymer distribution. The mobile phase was DMAc with 0.11% LiCl. The chromatographic conditions used were 50° C. in the column and detector compartments, 40° C. in the sample and injector compartment, a flow rate of 0.5 mL/min, and an injection volume of 100 μL. The software packages used for data reduction were Empower<sup>TM</sup> version 3 from Waters (calibration with broad glucan polymer standard) and Astra® version 6 from Wyatt (triple detection method with column calibration).

#### Example 1

#### Production of Gtf Enzyme 0874 (SEQ ID NO:2)

This Example describes preparing an N-terminally truncated version of a *Streptococcus sobrinus* gtf enzyme identified in GENBANK under GI number 450874 (SEQ ID NO:2, encoded by SEQ ID NO:1; herein referred to as "0874").

A nucleotide sequence encoding gtf 0874 was synthesized using codons optimized for protein expression in *E. coli* 45 (DNA2.0, Inc., Menlo Park, Calif.). The nucleic acid product (SEQ ID NO:1), encoding gtf 0874 (SEQ ID NO:2), was subcloned into pJexpress404® (DNA2.0, Inc.) to generate the plasmid construct identified as pMP57. This plasmid construct was used to transform *E. coli* TOP10 cells (Invitrogen, 50 Carlsbad, Calif.) to generate the strain identified as TOP10/pMP57.

Production of gtf 0874 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The 55 enzymatic activity of gtf 0874 is shown in Table 2 (see Example 18 below).

#### Example 2

#### Production of Gtf Enzyme 6855 (SEQ ID NO:4)

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 228476855 (SEQ ID 65 NO:4, encoded by SEQ ID NO:3; herein referred to as "6855").

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A nucleotide sequence encoding gtf 6855 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:3), encoding gtf 6855 (SEQ ID NO:4), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP53. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP53.

Production of gtf 6855 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 6855 is shown in Table 2 (see Example 18 below).

#### Example 3

#### Production of Gtf Enzyme 2379 (SEQ ID NO:6)

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 662379 (SEQ ID NO:6, encoded by SEQ ID NO:5; herein referred to as "2379")

A nucleotide sequence encoding gtf 2379 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:5), encoding gtf 2379 (SEQ ID NO:6), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP66. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP66.

Production of gtf 2379 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 2379 is shown in Table 2 (see Example 18 below).

#### Example 4

### Production of Gtf Enzyme 7527 (GtfJ, SEQ ID NO:8)

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 47527 (SEQ ID NO:8, encoded by SEQ ID NO:7; herein referred to as "7527" or "GtfJ").

A nucleotide sequence encoding gtf 7527 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:7), encoding gtf 7527 (SEQ ID NO:8), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP65. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP65

Production of gtf 7527 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 7527 is shown in Table 2 (see 60 Example 18 below).

#### Example 5

#### Production of Gtf Enzyme 1724 (SEQ ID NO:10)

This Example describes preparing an N-terminally truncated version of a *Streptococcus downei* gtf enzyme identified

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in GENBANK under GI number 121724 (SEQ ID NO:10, encoded by SEQ ID NO:9; herein referred to as "1724").

A nucleotide sequence encoding gtf 1724 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:9), encoding gtf 1724 (SEQ ID NO:10), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP52. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP52.

Production of gtf 1724 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 1724 is shown in Table 2 (see Example 18 below).

#### Example 6

#### Production of Gtf Enzyme 0544 (SEQ ID NO:12)

This Example describes preparing an N-terminally truncated version of a *Streptococcus mutans* gtf enzyme identified in GENBANK under GI number 290580544 (SEQ ID NO:12, encoded by SEQ ID NO:11; herein referred to as "0544").

A nucleotide sequence encoding gtf 0544 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:11), encoding gtf 0544 (SEQ ID NO:12), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP55. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP55.

Production of gtf 0544 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 0544 is shown in Table 2 (see Example 18 below).

#### Example 7

#### Production of Gtf Enzyme 5926 (SEQ ID NO:14)

This Example describes preparing an N-terminally truncated version of a *Streptococcus dentirousetti* gtf enzyme identified in GENBANK under GI number 167735926 (SEQ ID NO:14, encoded by SEQ ID NO:13; herein referred to as "5926").

A nucleotide sequence encoding gtf 5926 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:13), 50 encoding gtf 5926 (SEQ ID NO:14), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP67. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP67

Production of gtf 5926 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 5926 is shown in Table 2 (see Example 18 below).

#### Example 8

### Production of Gtf Enzyme 4297 (SEQ ID NO:16)

This Example describes preparing an N-terminally truncated version of a *Streptococcus oralis* gtf enzyme identified

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in GENBANK under GI number 7684297 (SEQ ID NO:16, encoded by SEQ ID NO:15; herein referred to as "4297").

A nucleotide sequence encoding gtf 4297 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:15), encoding gtf 4297 (SEQ ID NO:16), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP62. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP62.

Production of gtf 4297 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 4297 is shown in Table 2 (see Example 18 below).

#### Example 9

#### Production of Gtf Enzyme 5618 (SEQ ID NO:18)

This Example describes preparing an N-terminally truncated version of a *Streptococcus sanguinis* gtf enzyme identified in GENBANK under GI number 328945618 (SEQ ID NO:18, encoded by SEQ ID NO:17; herein referred to as "5618").

A nucleotide sequence encoding gtf 5618 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:17), encoding gtf 5618 (SEQ ID NO:18), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP56. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP56.

Production of gtf 5618 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 5618 is shown in Table 2 (see Example 18 below).

#### Example 10

#### Production of Gtf Enzyme 2765 (SEQ ID NO:20)

This Example describes preparing an N-terminally truncated version of a *Streptococcus* sp. gtf enzyme identified in GENBANK under GI number 322372765 (SEQ ID NO:20, encoded by SEQ ID NO:19; herein referred to as "2765").

A nucleotide sequence encoding gtf 2765 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:19), encoding gtf 2765 (SEQ ID NO:20), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP73. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP73.

Production of gtf 2765 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 2765 is shown in Table 2 (see 60 Example 18 below).

#### Example 11

#### Production of Gtf Enzyme 4700 (SEQ ID NO:22)

This Example describes preparing an N-terminally truncated version of a *Leuconostoc mesenteroides* gtf enzyme

identified in GENBANK under GI number 21654700 (SEQ ID NO:22, encoded by SEQ ID NO:21; herein referred to as "4700").

A nucleotide sequence encoding gtf 2765 was synthesized using codons optimized for protein expression in *E. coli* <sup>5</sup> (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:21), encoding gtf 4700 (SEQ ID NO:22), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP83. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/ <sup>10</sup> pMP83.

Production of gtf 4700 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 4700 is shown in Table 2 (see Example 18 below).

#### Example 12

Production of Gtf Enzyme 1366 (SEQ ID NO:24)

This Example describes preparing an N-terminally truncated version of a *Streptococcus criceti* gtf enzyme identified in GENBANK under GI number 146741366 (SEQ ID NO:24, encoded by SEQ ID NO:23; herein referred to as "1366").

A nucleotide sequence encoding gtf 1366 was synthesized using codons optimized for protein expression in *E. coli* <sup>30</sup> (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:23), encoding gtf 1366 (SEQ ID NO:24), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP86. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/ <sup>35</sup> pMP86.

Production of gtf 1366 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 1366 is shown in Table 2 (see 40 Example 18 below).

#### Example 13

Production of Gtf Enzyme 0427 (SEQ ID NO:26)

This Example describes preparing an N-terminally truncated version of a *Streptococcus sobrinus* gtf enzyme identified in GENBANK under GI number 940427 (SEQ ID NO:26, encoded by SEQ ID NO:25; herein referred to as "0427").

A nucleotide sequence encoding gtf 0427 was synthesized 55 using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:25), encoding gtf 0427 (SEQ ID NO:26), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP87. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP87.

Production of gtf 0427 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The 65 enzymatic activity of gtf 0427 is shown in Table 2 (see Example 18 below).

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Example 14

Production of Gtf Enzyme 2919 (SEQ ID NO:28)

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 383282919 (SEQ ID NO:28, encoded by SEQ ID NO:27; herein referred to as "2919").

A nucleotide sequence encoding gtf 2919 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:27), encoding gtf 2919 (SEQ ID NO:28), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP88. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP88.

Production of gtf 2919 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 2919 is shown in Table 2 (see Example 18 below).

#### Example 15

Production of Gtf Enzyme 2678 (SEQ ID NO:30)

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 400182678 (SEQ ID NO:30 encoded by SEQ ID NO:29; herein referred to as "2678").

A nucleotide sequence encoding gtf 2678 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:29), encoding gtf 2678 (SEQ ID NO:30), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP89. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP89.

Production of gtf 2678 by bacterial expression and determination of its enzymatic activity were performed following the procedures disclosed in the General Methods section. The enzymatic activity of gtf 2678 is shown in Table 2 (see Example 18 below).

#### Example 16

Production of Gtf Enzyme 2381 (SEQ ID NO:32)

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 662381 (SEQ ID NO:32 encoded by SEQ ID NO:31; herein referred to as "2381").

A nucleotide sequence encoding gtf 2381 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:31), encoding gtf 2381 (SEQ ID NO:32), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP96. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP96.

Production of gtf 2381 by bacterial expression and determination of its enzymatic activity were performed following

the procedures disclosed in the General Methods section. The enzymatic activity of gtf 2381 is shown in Table 2 (see Example 18 below).

#### Example 17

# Production of Gtf Enzyme 3929 (SEQ ID NO:34) and Additional Gtf Enzymes

This Example describes preparing an N-terminally truncated version of a *Streptococcus salivarius* gtf enzyme identified in GENBANK under GI number 387783929 (SEQ ID NO:34 encoded by SEQ ID NO:33; herein referred to as "3929").

A nucleotide sequence encoding gtf 3929 was synthesized using codons optimized for protein expression in *E. coli* (DNA2.0, Inc.). The nucleic acid product (SEQ ID NO:33), encoding gtf 3929 (SEQ ID NO:34), was subcloned into pJexpress404® to generate the plasmid construct identified as pMP97. This plasmid construct was used to transform *E. coli* TOP10 cells to generate the strain identified as TOP10/pMP97.

Production of gtf 3929 by bacterial expression and determination of its enzymatic activity were performed following 25 the procedures disclosed in the General Methods section. The enzymatic activity of gtf 3929 is shown in Table 2 (see Example 18 below).

Additional gtf enzymes were produced in a similar manner. Briefly, N-terminally truncated versions of enzymes identi- 30 fied in GENBANK under GI numbers 228476907 (a Streptococcus salivarius gtf, SEQ ID NO:36, herein referred to as "6907"), 228476661 (a Streptococcus salivarius gtf, SEQ ID NO:38, herein referred to as "6661"), 334280339 (a Streptococcus gallolyticus gtf, SEQ ID NO:40, herein referred to as 35 "0339"), 3130088 (a Streptococcus mutans gtf, SEQ ID NO:42, herein referred to as "0088"), 24379358 (a Streptococcus mutans gtf, SEQ ID NO:44, herein referred to as "9358"), 325978242 (a Streptococcus gallolyticus gtf, SEQ ID NO:46, herein referred to as "8242"), 324993442 (a Strep-40 tococcus sanguinis gtf, SEQ ID NO:48, herein referred to as "3442"), 47528 (a Streptococcus salivarius gtf, SEQ ID NO:50, herein referred to as "7528"), 322373279 (a Streptococcus sp. gtf, SEQ ID NO:52, herein referred to as "3279"), 170016491 (a Leuconostoc citreum gtf, SEQ ID NO:54, 45 herein referred to as "6491"), 228476889 (a Streptococcus salivarius gtf, SEQ ID NO:56, herein referred to as "6889"), 51574154 (a Lactobacillus reuteri gtf, SEQ ID NO:58, herein referred to as "4154"), and 322373298 (a Streptococcus sp. gtf, SEQ ID NO:59, herein referred to as "3298") were pre-50 pared and tested for enzymatic activity (Table 2, see Example 18 below).

#### Example 18

#### Production of Insoluble Glucan Polymer with Gtf Enzymes

This Example describes using the gtf enzymes prepared in 60 the above Examples to synthesize glucan polymer.

Reactions were performed with each of the above gtf enzymes following the procedures disclosed in the General Methods section. Briefly, gtf reaction solutions were prepared comprising sucrose (50 g/L), potassium phosphate buffer (pH  $\,$  65.5, 50 mM) and a gtf enzyme (2.5% extract by volume). After 24-30 hours at 22-25° C., insoluble glucan polymer product

was harvested by centrifugation, washed three times with water, washed once with ethanol, and dried at  $50^{\circ}$  C. for 24-30 hours.

Following the procedures disclosed in the General Methods section, the glycosidic linkages in the insoluble glucan polymer product from each reaction were determined by  $^{13}$ C NMR, and the DP, for each product was determined by SEC. The results of these analyses are shown in Table 2.

TABLE 2

Lin	Linkages and DP, of Glucan Produced by Various Gtf Enzymes											
	SEQ ID	Reducing Sugars	Insoluble Glucan	Glucar Link								
Gtf	NO.	Produced?	Produced?	%1,3	%1,6	$\mathrm{DP}_n$						
0874	2	yes	yes	100	0	60						
6855	4	yes	yes	100	0	440						
2379	6	yes	yes	37	63	310						
7527	8	yes	yes	100	0	440						
1724	10	yes	yes	100	0	250						
0544	12	yes	yes	62	36	980						
5926	14	yes	yes	100	0	260						
4297	16	yes	yes	31	67	800						
5618	18	yes	yes	34	66	1020						
2765	20	yes	yes	100	0	280						
4700	22	yes	no									
1366	24	yes	no									
0427	26	yes	yes	100	0	120						
2919	28	yes	yes	100	0	250						
2678	30	yes	yes	100	0	390						
2381	32	yes	no									
3929	34	yes	yes	100	0	280						
6907	36	yes	no									
6661	38	yes	no									
0339	40	yes	no									
0088	42	yes	no									
9358	44	yes	no									
8242	46	yes	no									
3442	48	yes	no									
7528	50	yes	no									
3279	52	yes	no									
6491	54	yes	no									
6889	56	yes	no									
4154	58	yes	no									
3298	59	yes	no									
none	na	no	no									

Several gtf enzymes produced insoluble glucan products (Table 2). However, only gtf enzymes 6855 (SEQ ID NO:4), 7527 (gtfJ, SEQ ID NO:8), 1724 (SEQ ID NO:10), 0544 (SEQ ID NO:12), 5926 (SEQ ID NO:14), 2765 (SEQ ID NO:20), 0427 (SEQ ID NO:26), 2919 (SEQ ID NO:28), 2678 (SEQ ID NO:30), and 3929 (SEQ ID NO:34) produced glucan comprising at least 50% alpha-1,3 linkages and having a DP<sub>n</sub> of at least 100. These enzymes are therefore suitable for producing glucan polymers for fiber applications.

Only gtfs 6855 (SEQ ID NO:4), 7527 (gtfJ, SEQ ID NO:8), 1724 (SEQ ID NO:10), 5926 (SEQ ID NO:14), 2765 (SEQ ID NO:20), 0427 (SEQ ID NO:26), 2919 (SEQ ID NO:28), 2678 (SEQ ID NO:30), and 3929 (SEQ ID NO:34) produced glucan polymer comprising 100% alpha-1,3 linkages and having a DP<sub>n</sub> of at least 100. These results, in which only nine out of thirty gtfs were able to produce glucan with 100% alpha-1,3 linkages and a DP<sub>n</sub> of at least 100, indicate that not all gtf enzymes are capable of producing high molecular weight, insoluble glucan with a high level of alpha-1,3 linkages. Fewer gtf enzymes were able to produce glucan polymer comprising 100% alpha-1,3 linkages and having a DP<sub>n</sub> of at least 250.

Thus, gtf enzymes capable of producing glucan polymer comprising 100% alpha-1,3 linkages and a  $DP_n$  of at least 100 were identified. These enzymes can be used to produce glucan suitable for producing fibers.

SEQUENCE LISTING

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Asp Asn Pro Ser Asp Trp Tyr Tyr Phe Gly Val Glu Phe Ala Ly	λa
Leu Thr Gly Leu Gln Lys Ile Gly Gln Gln Thr Leu Tyr Phe As 1085 1090 1095	ap
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Val Thr Leu Ala Asp Lys Ser Ile Arg Tyr Phe Asp Ala Asp Se 1175 1180 1185	er
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Gly Ser Gln Asn Glu Trp Tyr Tyr Phe Asp Gln Ala Gly Lys A 1265 1270 1275	la
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Gln Asp Gly Lys Gln Val Lys Gly Lys Val Val Asp Val Asn G 1295 1300 1305	ly
Val Ser Arg Tyr Phe Asp Ala Asn Ser Gly Asp Met Ala Arg Sc 1310 1315 1320	er

-continued

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<sup>&</sup>lt;212> TYPE: PRT <213> ORGANISM: Streptococcus sanguinis

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Lys 465	Lys	Ala	Phe	Glu	Ile 470	Tyr	Asn	Lys	Asp	Met 475	Leu	Ser	Ser	Asp	Lys 480
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ГÀа	Ser 610	Ala	ГÀа	Leu	Asp	Val 615	Glu	Met	Gly	Lys	Ile 620	His	Ala	Asn	Gln
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171 172

Met Asn

<210> SEQ ID NO 29

<211> LENGTH: 4026

<212> TYPE: DNA

<213> ORGANISM: Streptococcus salivarius

<400> SEQUENCE: 29

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<210> SEQ ID NO 30
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<sup>&</sup>lt;211> LENGTH: 1341

<sup>&</sup>lt;212> TYPE: PRT

<sup>&</sup>lt;213> ORGANISM: Streptococcus salivarius

<sup>&</sup>lt;400> SEQUENCE: 30

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Thr	Pro 210	Trp	Ala	Asn	Ser	Asn 215	Tyr	Arg	Leu	Leu	Asn 220	Arg	Thr	Ala	Thr
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Ala	His	Asp 435	Asn	Asn	Val	Gln	Asp 440	Ile	Ile	Ala	Glu	Ile 445	Ile	Lys	Lys
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See Ser Gly Arg Asp Tyr Cln Ala Gln Tyr Gly Gly Glu Phe Leu Ala 865 Ser Gly Arg Asp Tyr Pro Lys Met Phe Thr Glu Asn Met Ile Ser 895 Ser Ser Ser Gly Lys Pyr Pyr Lys Met Phe Thr Glu Asn Met Ile Ser 895 Ser	_	050					OFF					0.60				
Second   S		850					855					860				
### Separation of the properties of the properti		Ser	Gly	Arg	Asp		Gln	Ala	Gln	Tyr		Gly	Glu	Phe	Leu	
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930 935 946  Asm Phe He Pro Leu Gin Leu Thr Gly Asm Giu Lys Ala Val Thr Gly 945  Phe Ser Asm Asp Gly Lys Gly He Thr Phe Asm Gly Asm Thr Tyr Phe 950  Asm Ala Lys Ser Ala Phe Val Thr 985  Gln Ala Lys Ser Ala Phe Val Thr 985 Gly Glu Tyr Ser Pro Asm Gly 1000  Asp Ala Arg Gly His Met Val Thr Asm Gly Glu Tyr Ser Pro Asm Gly 1000  Lys Asp Val Tyr Arg Phe Leu Pro Asm Gly Ile Met Leu Ser Asm 1010  Ala Phe Tyr Val Asp Ala Asm Gly Asm Thr Tyr Leu 1025  Glu Thr Asm Glu Gly Ser Gly Asm Gly Tyr Thr Lys 1005  Glu Thr Asp Lys Asp Gly Asm Glu Ser Lys Val Val Lys Phe Arg 1066  Tyr Phe Thr Asm Glu Gly Val Met Ala Lys Gly Leu Thr 1086  Asp Lys Asp Gly Asm Thr Tyr Lys Gly Gly Gly Asm Thr Tyr Tyr Phe Gly Asm 1005  Asp Gly Ser Thr Gln Tyr Phe Gly Gly Asm Thr Tyr Tyr Phe Glu Ala 1110  His Thr Gly Asm Ala Ile Lys Asm Thr Tyr Tyr Tyr Phe Glu Ala 1110  Glu Val Ile Asm Gly Glu Lys Lys Thr Tyr Tyr Tyr The Glu Ala 1110  Gli Val Ile Asm Gly Gly Lau Lys Asm Ala Ala Asm Gly Val Ala Ala Ile Lys Asm Ala Ala Asm Glu Phe Phe 1155  Gli Val Ile Asm Gly Gly Val Lys Thr Tyr Phe Asm Glu Asp Gly Ser Iliso  Thr Thr Asm Gly Gly Ser Glu Asm Gly Val Ala Ala Ala Thr Tyr Lys 1165  Thr Thr Asm Gly Gly Ser Gly Asm Ala Ile Lys Asm Ala Asp Gly Firs Thr Gly Ala 1115  Thr Thr Asp Gly Asm Val Trp Tyr Tyr Ala Gly Ala Asp Gly Lys Iliso  Thr Val Thr Gly Ala Glu Val Lys Gly Gly Leu Val Thr Asm Glu Phe Phe 1120  Thr Val Thr Gly Ala Glu Val Lys Gly Gly Leu Val Val Lys Asm Ala Asp Gly Lys Iliso  Thr Thr Asp Gly Asm Val Trp Tyr Tyr Ala Gly Ala Asp Gly Lys Iliso  Thr Thr Asp Gly Asm Val Trp Tyr Tyr Ala Gly Ala Asp Gly Lys Iliso  Thr Thr Asp Gly Asm Val Trp Tyr Tyr Ala Gly Ala Asp Gly Lys Iliso  Thr Val Thr Gly Ala Gln Val Lys Gly Gly Gly Val Val Lys Asm Ala Ala Thr Gly Asm Ala Iliso  Thr Thr Asp Gly Asm Clu Tyr Ser Lys Tyr Asp Ala Ala Thr Gly Glu Arg Leu Iliso  Thr Asm Glu Phe Phe Thr Thr Gly Asp Asm Asm Trp Tyr Tyr Ile	Lys	Tyr		Asn	Gly	Thr	Asn		Leu	Asp	Arg	Gly		_	Tyr	Val
945	Leu		Asp	Glu	Ala			Lys	Tyr	Phe	Thr		Thr	Lys	Glu	. Gly
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Agp   Ala   Arg   Gly   His   Met   Val   Thr   Asn   Gly   Glu   Tyr   Ser   Pro   Asn   Gly   Lys   Asn   1015   Pro   Asn   Gly   Ile   Met   Leu   Ser   Asn   Gly   1005   Pro   Asn   Gly   1005   Pro   Asn   Gly   1005   Pro   Asn   Gly   1005   Pro   Asn   Gly   Asn   Inr   Tyr   Leu   Leu   Tyr   Asn   Tyr   1035   Pro   Asn   Tyr   Pro   1035   Pro   Asn   Tyr   Pro   1065   Pro   Asn   Tyr   Pro   1066   Pro   Pro   Asn   Tyr   Pro   1065   Pro   Asn   Tyr   Pro   1066   Pro   Pro   Asn   Tyr   Pro   1066   Pro   Pro   Asn   Tyr   Pro   Asn   Tyr   Pro   1066   Pro   Pro   Asn   Tyr   Pro   Asn   Tyr   Pro   Pro   Asn   Tyr   Pro   Pro   Asn   Tyr   Pro	Phe	Ser	Asn	Asp		Lys	Gly	Ile	Thr		Phe	Gly	Thr	Ser		
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1010	Asp	Ala		Gly	His	Met	Val			n Gly	y Glu	1 Ту:			ro A	sn Gly
Lys Gly 1000	ГÀа			. Туз	r Arg	g Phe			ro As	sn G	ly II			Leu	Ser	Asn
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Asp Lys Leu Ala Thr Tyr Lys Gly Lys Thr Tyr Tyr Phe Glu Ala 1100    His Thr Gly Asn Ala Ile Lys Asn Thr Trp Arg Asn Ile Asp Gly 1115    Lys Trp Tyr His Phe Asp Glu Asn Gly Val Ala Ala Thr Gly Ala 1130    Gln Val Ile Asn Gly Gln Lys Leu Tyr Phe Asn Glu Asp Gly Ser 1145    Gln Val Lys Gly Gly Val Val Lys Asn Ala Asp Gly Thr Tyr Ser 1160    Lys Tyr Lys Glu Gly Ser Gly Glu Leu Val Thr Asn Glu Phe Phe 1175    Thr Thr Asp Gly Asn Val Trp Tyr Tyr Ala Gly Ala Asp Gly Lys 1190    Thr Val Thr Gly Ala Gln Val Ile Asn Gly Gly Ile Asn Gly Gln His Leu Tyr Phe 1210    Lys Glu Asp Gly Ser Gln Val Lys Gly Gly Val Ile Asn Gly Gln His Leu Tyr Phe 1225    Lys Glu Asp Gly Ser Gln Val Lys Gly Gly Val Val Lys Gly Gly Val Val Lys Asn Ala Asp Gly Thr Tyr Asn Ala Lys Asn Ala Lys Asn Ala Lys Gly Thr Tyr Asn Asp Gly Glu Arg Leu Lys Gly Thr Tyr Ser Lys Tyr Asp Ala Ala Thr Gly Glu Arg Leu Lys Gly Thr Asn Glu Phe Phe Thr Thr Gly Asp Asn Asn Trp Tyr Tyr Ile	Tyr			: Asr	n Glu	ı Gly			et A	la Ly	ys Gl	_		Thr	Val	Ile
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	Asp			туз	s Ser	: Lys			sp Al	La A	la Th			Glu	Arg	Leu
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Gln Trp Tyr Tyr Phe Asp Lys Asn Gly Asn Met Val Thr Gly Glu (930 935 940	Gln
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Leu His Asp Met Tyr Gly Thr Thr Phe Tyr Phe Asp Glu Lys Th	han
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Gly Ile Gln Ala Lys Asp Lys Phe Ile Arg Phe Ala Asp Gly Ast 1025 1030 Thr Arg Tyr Phe Ile Pro Asp Thr Gly Asn Leu Ala Val Asn As	rg rg
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Gly Ile Gln Ala Lys Asp Lys Phe Ile Arg Phe Ala Asp Gly Asp 1035  Thr Arg Tyr Phe Ile Pro Asp 1045  Phe Ala Gln Asn Pro Glu Asn Lys Ala Trp Tyr Tyr Leu Asp Sc 1055  Asn Gly Tyr Ala Val Thr Gly Leu Gln Thr Ile Asn Gly Lys Girl 1070  Tyr Tyr Phe Asp Asn Glu Gly 1090  Thr Ile Asn Asn Asn Gln Arg Tyr Phe Leu Asp Gly Asp Scr Gly Girl 1100  The Ala Pro Ser Arg Phe Val Thr Glu Asn Asn Lys Trp Tyr	rg er ln al lu yr
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Ala	Gln 1070		a Lys	s Lys	Gly	Glu 107		ne T	yr Tl	nr T		080 ab	Gly :	Ser '	Thr
Trp	Phe		r Arg	g Asp	) Ala	Ala		ly L	ys A	sn V		nr 095	Gly A	Ala i	Leu
Thr	Leu 1100	_	Gly	/ His	s Glu	Туз 110	_	yr Pl	ne A:	rg A		sn 110	Gly A	Ala (	Gln

Val Lys Gly Glu Phe Val Thr Glu Asn Gly Lys 11e Ser Tyr Tyr 1115 Thr Val Asp Asn Gly Tyr Lys Val Lys Asp Lys Phe Phe Glu Val 1135
Asn Gly Lys Trp Tyr His Ala Asp Lys Asp Gly Asp Leu Ala Thr 1145  Gly Arg Gln Thr Ile Asp His Leu Asn Tyr Tyr Phe Asn Ala Asp 1165  Gly Arg Gln Thr Ile Asp His Leu Asn Tyr Tyr Phe Asn Ala Asp 1165  Gly Arg Gln Thr Ile Asp His Leu Asn Tyr Tyr Phe Asn Ala Asp 1170  Gly Ser Gln Val Lys Ser Asp Phe Phe Thr Leu Asp Gly Gly Lys 1175  Thr Trp Tyr Tyr Ala Lys Asp Asn Gly Glu Ile Val Thr Gly Ala 1190  Tyr Ser Val Arg Gly Lys Asn Tyr Tyr Phe Lys Glu Asp Gly Ser Leu Ser 1220  Gln Val Lys Gly Asp Phe Val Lys Asn Ala Asp Gly Ser Leu Ser 1220  Tyr Tyr Asp Lys Asp Ser Gly Glu Arg Leu Asn Asn Arg Phe Leu 1245  Thr Thr Gly Asn Asn Val Trp Tyr Tyr Phe Lys Asp Gly Lys Ala 1250  This Leu Gly Arg Gln Asn Ile Asp Gly Lys Glu Tyr Tyr Phe Asp 1275  His Leu Gly Arg Gln Val Lys Gly Ser Pro Ile Ser Thr Pro Lys 1285  Gly Val Glu Tyr Tyr Glu Ser Val Leu Gly Glu Arg Val Thr Asn 1295  Gly Val Glu Tyr Tyr Glu Ser Val Leu Gly Glu Arg Phe Asp Glu 1310  Thr Trp Ile Thr Phe Gln Asp Gly Lys Thr Val Phe Phe Asp Glu 1310  Asn Gly Tyr Ala Asp Phe Asp Lys 1320 <pre></pre>
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Gln Val Lys Gly Asp Phe Val Lys Asn Ala Asp Gly Ser Leu Ser 1220  Tyr Tyr Asp Lys Asp Ser Gly Glu Arg Leu Asn Asn Arg Phe Leu 1235  Thr Thr Gly Asn Asn Val Trp Tyr Tyr Phe Lys Asp Gly Lys Ala 1255  Thr Thr Gly Arg Gln Asn Ile Asp Gly Lys Glu Tyr Tyr Phe Asp 1260  Val Thr Gly Arg Gln Val Lys Gly Ser Pro Ile Ser Thr Pro Lys 1280  Gly Val Glu Tyr Tyr Glu Ser Val Leu Gly Glu Arg Val Thr Asn 1295  Thr Trp Ile Thr Phe Gln Asp Gly Lys Thr Val Phe Phe Asp Glu 1310  Asn Gly Tyr Ala Asp Phe Asp 1325  Asn Gly Tyr Ala Asp Phe Asp 1330  **C210> SEO ID NO 37  **C211> LENGTH: 3918  **C212> TYPE: DNA  **C212> TYPE: DNA  **C400> SEQUENCE: 37  atgattgacg gcaaacagta ttatgtagag aacggtgtg ttaagaagaa tacggcgatt 60  gaactggatg gccgtctgta ttactttgac gaaaccggtg caatggttga tcaatctaag 120  ccgctgtatc gcgcggatge aatcccgaac aactctatct acgcagttta caaccaggct 180  tacgaccaca gcagcaagag ctttgaacac ctggacaact ttctgacgg cgataagctgg 240  tacgacccac acagggttt gaacaacg cagaaatcgg cagaattga cacccgac gagagagac 300  tatcgtcctt tgctgatgac gtggtggccg gataaagtca cgccccgac gagagagac 360  tatatgtccc aacagggtt tggtaacaag acctacacca cggatattag tagacagac 420  ctggcggcag cggcggaaac ggttcagcgt ggcatcgaag aggctattg tcgtgagggt 480  aatacgacgt ggctggtaa gtgtgtggcg gatacaagac ctgcaagtca ctacctgaac 360  tatatgtccc aacagggctt tggtaacaag gacttcacca aacccagc gggctggaat 540  agcgagagcg aagataatct gctggtcggt aacgatcatc tgcaagtgt gtgcactgacg 660  cttctgaacca atagcacca cggacaatac ccgcaaatca caccagc ggctggaat 540  agcgagagcg aagataatct gctggtcggt aacgactac tcgcaagtgt gcactgacg 660  cttctgaacca aacacggacc ccgcaaatac caccacacacacacacacacacacacacacac
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Ala Glu Lys Asn Thr A 165	sn Trp Leu Arg	Gln Thr Ile 170	Ser Ala Phe	Val
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His Leu Gln Lys Gly A	la Leu Leu Tyr	Ser Asn Asn	Ser Lys Leu	Thr
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<sup>&</sup>lt;212> TYPE: PRT

<sup>&</sup>lt;213> ORGANISM: Streptococcus salivarius

<sup>&</sup>lt;400> SEOUENCE: 50

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Pro 65	Glu	Ala	Thr	ГÀа	Glu 70	Ala	Aap	Lys	Arg	Thr 75	Asn	Thr	ГÀа	Glu	Ala 80
Asp	Val	Leu	Thr	Pro 85	Ala	ГЛа	Glu	Thr	Asn 90	Ala	Val	Glu	Thr	Ala 95	Thr
Thr	Thr	Asn	Thr 100	Gln	Ala	Thr	Ala	Glu 105	Ala	Ala	Thr	Thr	Ala 110	Thr	Thr
Ala	Asp	Val 115	Ala	Val	Ala	Ala	Val 120	Pro	Asn	Lys	Glu	Ala 125	Val	Val	Thr
Thr	Asp 130	Ala	Pro	Ala	Val	Thr 135	Thr	Glu	Lys	Ala	Glu 140	Glu	Gln	Pro	Ala
Thr 145	Val	Lys	Ala	Glu	Val 150	Val	Asn	Thr	Glu	Val 155	Lys	Ala	Pro	Glu	Ala 160
Ala	Leu	Lys	Asp	Ser 165	Glu	Val	Glu	Ala	Ala 170	Leu	Ser	Leu	Lys	Asn 175	Ile
ГÀв	Asn	Ile	Asp 180	Gly	Lys	Tyr	Tyr	Tyr 185	Val	Asn	Glu	Asp	Gly 190	Ser	His
ГÀа	Glu	Asn 195	Phe	Ala	Ile	Thr	Val 200	Asn	Gly	Gln	Leu	Leu 205	Tyr	Phe	Gly
ГÀа	Asp 210	Gly	Ala	Leu	Thr	Ser 215	Ser	Ser	Thr	Tyr	Ser 220	Phe	Thr	Pro	Gly
Thr 225	Thr	Asn	Ile	Val	Asp 230	Gly	Phe	Ser	Ile	Asn 235	Asn	Arg	Ala	Tyr	Asp 240
Ser	Ser	Glu	Ala	Ser 245	Phe	Glu	Leu	Ile	Asp 250	Gly	Tyr	Leu	Thr	Ala 255	Asp
Ser	Trp	Tyr	Arg 260	Pro	Ala	Ser	Ile	Ile 265	Lys	Asp	Gly	Val	Thr 270	Trp	Gln
Ala	Ser	Thr 275	Ala	Glu	Asp	Phe	Arg 280	Pro	Leu	Leu	Met	Ala 285	Trp	Trp	Pro
Asn	Val 290	Asp	Thr	Gln	Val	Asn 295	Tyr	Leu	Asn	Tyr	Met 300	Ser	ГÀа	Val	Phe
Asn 305	Leu	Asp	Ala	Lys	Tyr 310	Ser	Ser	Thr	Asp	Lys 315	Gln	Glu	Thr	Leu	Lys 320
Val	Ala	Ala	Lys	Asp 325	Ile	Gln	Ile	Lys	Ile 330	Glu	Gln	Lys	Ile	Gln 335	Ala
Glu	Lys	Ser	Thr 340	Gln	Trp	Leu	Arg	Glu 345	Thr	Ile	Ser	Ala	Phe 350	Val	Lys
Thr	Gln	Pro 355	Gln	Trp	Asn	Lys	Glu 360	Thr	Glu	Asn	Tyr	Ser 365	Lys	Gly	Gly
Gly	Glu 370	Asp	His	Leu	Gln	Gly 375	Gly	Ala	Leu	Leu	Tyr 380	Val	Asn	Asp	Ser
Arg 385	Thr	Pro	Trp	Ala	Asn 390	Ser	Asp	Tyr	Arg	Arg 395	Leu	Asn	Arg	Thr	Ala 400

Asp         Fro         Asp         Leu         Leu         Asp         Asp         Leu         Leu         Asp         Asp <th>Thr</th> <th>Asn</th> <th>Gln</th> <th>Thr</th> <th>Gly 405</th> <th>Thr</th> <th>Ile</th> <th>Asp</th> <th>ГÀз</th> <th>Ser 410</th> <th>Ile</th> <th>Leu</th> <th>Asp</th> <th>Glu</th> <th>Gln 415</th> <th>Ser</th>	Thr	Asn	Gln	Thr	Gly 405	Thr	Ile	Asp	ГÀз	Ser 410	Ile	Leu	Asp	Glu	Gln 415	Ser
143   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140   140	Asp	Pro	Asn		Met	Gly	Gly	Phe	-	Phe	Leu	Leu	Ala		Asp	Val
Phe   Asp   Cly   Ile   Arg   Val   Asp   Ala   Val   Asp   Asp   Val   Asp   Ala   Asp   Ala   Asp   Ala   Asp   Ala   Ala	Asp	Leu		Asn	Pro	Val	Val		Ala	Glu	Gln	Leu		Gln	Ile	His
480 Leu Gln Leu Tyr Thr Asn Tyr Phe Arg Glu Tyr Tyr Gly Val Asn Lys Asn Soo Soo Soo Soo Soo Soo Soo Soo Soo So	Tyr		Met	Asn	Trp	Gly		Ile	Val	Met	Gly		Lys	Asp	Ala	Asn
See   Glu   Ala   Asn   Asn		Asp	Gly	Ile	Arg		Asp	Ala	Val	Asp		Val	Asp	Ala	Asp	
Sol	Leu	Gln	Leu	Tyr		Asn	Tyr	Phe	Arg		Tyr	Tyr	Gly	Val		Lys
Met         Glu         Asn         Lys         Gln         Arg         Leu         Leu         Leu         Phe         Ser         Leu         Ala         Lys         Pho         Ala         Leu         Pho         Ser         Leu         Ala         Lys         Pho         Ser         Pro         Ala         Leu         Pho         Ser         Leu         Ala         Lys         Pho         Ser         Lys         Ala         Tyr         Ala         Pho         Ser         Pho         Ser         Lys         Ala         Tyr         Ala         Pho         Ser         Ala         Lys         Ala         Tyr         Ala         Ala         Ala         Pho         Ser         Blu         Lys         Ala         Ala <td>Ser</td> <td>Glu</td> <td>Ala</td> <td></td> <td>Ala</td> <td>Leu</td> <td>Ala</td> <td>His</td> <td></td> <td>Ser</td> <td>Val</td> <td>Leu</td> <td>Glu</td> <td></td> <td>Trp</td> <td>Ser</td>	Ser	Glu	Ala		Ala	Leu	Ala	His		Ser	Val	Leu	Glu		Trp	Ser
S30	Leu	Asn	_	Asn	His	Tyr	Asn	_	Lys	Thr	Asp	Gly		Ala	Leu	Ala
556         556         556         556         569         610         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640         640 <td>Met</td> <td></td> <td>Asn</td> <td>Lys</td> <td>Gln</td> <td>Arg</td> <td></td> <td>Ala</td> <td>Leu</td> <td>Leu</td> <td>Phe</td> <td></td> <td>Leu</td> <td>Ala</td> <td>Lys</td> <td>Pro</td>	Met		Asn	Lys	Gln	Arg		Ala	Leu	Leu	Phe		Leu	Ala	Lys	Pro
Ser         Lys         Ala         Tyr         Asn         Glu         Asp         Gly         Thr         Val         Lys         Gln         Ser         Thr         Ile         Gly         Ser         Gly         Thr         Lys         Gly         Asp         Ala         Lys         Gly         Asp         Ala         Lys         Gly         Asp         Ala         Lys         Gly         Asp         Ala         Ser         Asp         Ala         Ser         Asp         Ala         Asp         Asp <td></td> <td>Lys</td> <td>Glu</td> <td>Arg</td> <td>Thr</td> <td></td> <td>Ala</td> <td>Val</td> <td>Ser</td> <td>Pro</td> <td></td> <td>Tyr</td> <td>Asn</td> <td>Asn</td> <td>Thr</td> <td></td>		Lys	Glu	Arg	Thr		Ala	Val	Ser	Pro		Tyr	Asn	Asn	Thr	
Lys         Tyr         Asn Sys         Syr         Gly Sys         Asp Sys         Ala Sys <td>Asn</td> <td>Thr</td> <td>Thr</td> <td>Gln</td> <td>_</td> <td>Asp</td> <td>Glu</td> <td>Lys</td> <td>Thr</td> <td>_</td> <td>Trp</td> <td>Ile</td> <td>Asn</td> <td>Lys</td> <td>_</td> <td>Gly</td>	Asn	Thr	Thr	Gln	_	Asp	Glu	Lys	Thr	_	Trp	Ile	Asn	Lys	_	Gly
Arg Ala His Asp Asn Asn Val Gln Asp Ile Ile Ala Glu Ile Ile Lys Glu Ile Asn Pro Lys Glu Ile Ile Asn Asn Asn Ile Tyr Asn Lys Asp Met Leu Ser Ser Asp Glu Ile	Ser	Lys	Ala		Asn	Glu	Asp	Gly		Val	Lys	Gln	Ser		Ile	Gly
610	Lys	Tyr		Glu	ГЛа	Tyr	Gly	_	Ala	Ser	Gly	Asn	_	Val	Phe	Ile
625	Arg		His	Asp	Asn	Asn		Gln	Asp	Ile	Ile		Glu	Ile	Ile	Lys
Company   Comp		Glu	Ile	Asn	Pro		Ser	Asp	Gly	Phe		Ile	Thr	Asp	Ala	
660 665 767 670  Gln Asn Met Glu Thr Ile Thr Arg Val Tyr Tyr Gly Asp Leu Tyr Thr 680 789 Asp Asp Gly His Tyr Met Gly Thr Lys Ser Tyr Tyr 705 Asn Leu Met Lys Ser Arg Ile Lys Tyr Val Ser Gly Gly Gln Ala 720  Gln Arg Ser Tyr Tyr Leu Pro Thr Asp Gly Lys Met Asp Asn Ser Asp 735  Val Glu Leu Tyr Arg Arg Thr Asn Glu Val Tyr Thr Ser Val Asp Tyr Gly 755  Lys Asp Ile Met Thr Ala Asn Asp Thr Glu Gly Ser Gly Ser Arg Arg Tyr Arg Tyr Arg Arg Tyr Arg Tyr Asp Tyr Gly 755  Asp Gln Ser Ala Lys Leu Asn Val Glu Met Gly Thr Ala Asp Gly Ile His Asp Asn Asp Tyr	Met	Lys	Gln	Ala		Glu	Ile	Tyr	Asn		Asp	Met	Leu	Ser		Asp
Asp       Asp 690       Gly His Tyr       Met 695       Lys Ser Pro 700       Tyr Tyr Asp Thr Ile         Val Asn Leu Met Lys 700       Asn Leu Met Lys 710       Asn Ile Lys Tyr 715       Asn Ile Lys 700       Asn Asp 700       Asp 700 <t< td=""><td>Lys</td><td>Lys</td><td>Tyr</td><td></td><td>Leu</td><td>Asn</td><td>Asn</td><td>Ile</td><td></td><td>Ala</td><td>Ala</td><td>Tyr</td><td>Ala</td><td></td><td>Met</td><td>Leu</td></t<>	Lys	Lys	Tyr		Leu	Asn	Asn	Ile		Ala	Ala	Tyr	Ala		Met	Leu
Val       Asn       Leu       Met       Lys       Ser       Arg       Ile       Lys       Tyr       Val       Ser       Gly       Gly       Gln       Ala         Gln       Arg       Ser       Tyr       Trp       Leu       Pro       Thr       Asp       Gly       Lys       Met       Asp       Asp       Asp       Asp       Gly       Lys       Met       Asp       Asp       Asp       Asp       Gly       Lys       Met       Asp       Asp       Asp       Thr       Ser       Val       Arg       Tyr       Gly       Asp       Asp       Asp       Thr       Ser       Val       Arg       Tyr       Arg       Asp       Asp       Asp       Thr       Ser       Val       Arg       Tyr       Ser       Arg       Tyr       Ser       Arg       Tyr       Ser       Arg       Arg       Tyr       Arg       Arg       Arg       Tyr       Arg       Arg       Arg       Tyr       Arg       A	Gln	Asn		Glu	Thr	Ile	Thr	_	Val	Tyr	Tyr	Gly	_	Leu	Tyr	Thr
710 715 720  Gln Arg Ser Tyr Trp Leu Pro Thr Asp Gly Lys Met Asp Asn Ser Asp 735  Val Glu Leu Tyr Arg Arg Thr Asn Glu Val Tyr Thr Ser Val Arg Tyr Gly 750  Lys Asp Ile Met Thr Ala Asn Asp Thr Glu Gly Ser Lys Tyr Ser Arg 755  Thr Ser Gly Gln Val Thr Leu Val Ala Asn Asn Pro Lys Leu Asn Leu 770  Asp Gln Lys Tyr Arg Ala Leu Ile Val Gly Thr Ala Asp Gly Ile His Ala Asn 800  Gln Lys Tyr Arg Ala Leu Ile Val Gly Thr Ala Asp Gly Ile Lys Asn	Asp		Gly	His	Tyr	Met		Thr	Lys	Ser	Pro		Tyr	Asp	Thr	Ile
Val       Glu       Leu       Tyr Arg       Thr Asn Glu       Val Tyr Thr Ser Val Arg Tyr Gly 750         Lys       Asp 755       Thr Ala Asn Asp 760       Thr Glu Gly Ser Lys 765       Tyr Ser Arg 765         Thr Ser Gly Gln Val Thr Leu Val Ala Asn 775       Asp 775       Asp 775         Asp 80       Gln Ser Ala Lys Leu Asn 770       Yal Gly Met 779       Gly Lys Leu Asn 800         Gln Lys Tyr Arg Ala Leu Ile Val Gly Thr Ala Asp 61y Ile Lys Asn		Asn	Leu	Met	Lys		Arg	Ile	Lys	Tyr		Ser	Gly	Gly	Gln	
Lys Asp 750       745       750         Lys Asp 11e Met 757       755       760       760       760       760       760       765       765       777       765       765       765       767       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765       765	Gln	Arg	Ser	Tyr		Leu	Pro	Thr	Asp		Lys	Met	Asp	Asn		Asp
Thr Ser Gly Gln Val Thr Leu Val Ala Asn Asn Pro Lys Leu Asn Leu 770	Val	Glu	Leu	_	Arg	Thr	Asn	Glu		Tyr	Thr	Ser	Val	_	Tyr	Gly
Asp Gln Ser Ala Lys Leu Asn Val Glu Met Gly Lys Ile His Ala Asn 800  Gln Lys Tyr Arg Ala Leu Ile Val Gly Thr Ala Asp Gly Ile Lys Asn	ГЛа	Asp		Met	Thr	Ala	Asn	_	Thr	Glu	Gly	Ser	_	Tyr	Ser	Arg
785 790 795 800 Gln Lys Tyr Arg Ala Leu Ile Val Gly Thr Ala Asp Gly Ile Lys Asn	Thr		Gly	Gln	Val	Thr		Val	Ala	Asn	Asn		Lys	Leu	Asn	Leu
		Gln	Ser	Ala	Lys		Asn	Val	Glu	Met		Lys	Ile	His	Ala	
	Gln	Lys	Tyr	Arg		Leu	Ile	Val	Gly		Ala	Asp	Gly	Ile		Asn

Phe	Thr	Ser	Asp 820	Ala	Asp	Ala	Ile	Ala 825	Ala	Gly	Tyr	Val	830 Tàa	Glu	Thr
Asp	Ser	Asn 835	Gly	Val	Leu	Thr	Phe 840	Gly	Ala	Asn	Asp	Ile 845	-	Gly	Tyr
Glu	Thr 850	Phe	Asp	Met		Gly 855	Phe	Val	Ala	Val	Trp 860	Val	Pro	Val	Gly
Ala 865	Ser	Asp	Asn	Gln	Asp 870	Ile	Arg	Val	Ala	Pro 875	Ser	Thr	Glu	Ala	880 Lys
Lys	Glu	Gly	Glu	Leu 885	Thr	Leu	Lys	Ala	Thr 890	Glu	Ala	Tyr	Asp	Ser 895	Gln
Leu	Ile	Tyr	Glu 900	Gly	Phe	Ser	Asn	Phe 905	Gln	Thr	Ile	Pro	Asp 910	Gly	Ser
Asp	Pro	Ser 915	Val	Tyr	Thr	Asn	Arg 920	Lys	Ile	Ala	Glu	Asn 925		Asp	Leu
Phe	Lys 930	Ser	Trp	Gly	Val	Thr 935	Ser	Phe	Glu	Met	Ala 940	Pro	Gln	Phe	Val
Ser 945	Ala	Asp	Asp	Gly	Thr 950	Phe	Leu	Asp	Ser	Val 955	Ile	Gln	Asn	Gly	Tyr 960
Ala	Phe	Ala	Asp	Arg 965	Tyr	Asp	Leu	Ala	Met 970	Ser	Lys	Asn	Asn	Lys 975	-
Gly	Ser	Lys	Glu 980	Asp	Leu	Arg	Asp	Ala 985	Leu	Lys	Ala	Leu	His	Lys	Ala
Gly	Ile	Gln 995	Ala	Ile	Ala	Asp	Trp		l Pro	o Asl	9 Gl:		е Т 05	yr G	ln Leu
Pro	Gly 1010		Glu	ı Val	. Val	Th:		la Ti	nr Ai	rg Tl		sp 020	Gly .	Ala	Gly
Arg	Lys 1025		e Ala	a Asp	Ala	Ile 103		le A	ap H:	is Se		eu 035	Tyr	Val	Ala
Asn	Ser 1040		s Sei	Ser	Gly	Lys 104		вр Т	yr G	ln Ai		ys 050	Tyr	Gly	Gly
Glu	Phe 1055		ı Ala	ı Glu	ı Leu	Lys 106		la L	ys Ty	yr Pi		lu 065	Met	Phe	Lys
Val	Asn 1070		: Ile	e Ser	Thr	Gl <sub>y</sub>		ys P:	ro I	le A:		080 sp	Ser '	Val	Lys
Leu	Lys 1085		n Trp	Lys	. Ala	Glu 109		yr Pl	ne As	sn G		hr 095	Asn '	Val	Leu
Glu	Arg 1100		/ Val	. Gly	y Tyr	Va]		eu Se	er As	sp Gi		la 110	Thr	Gly	Lys
Tyr	Phe 1115		· Val	. Thr	. Lys	Glu 112		ly A	sn Pl	ne II		ro 125	Leu	Gln	Leu
Thr	Gly 1130		Glu	ı Lys	. Val	Ile 113		nr G	ly Pl	ne Se		er 140	Asp	Gly	Lys
Gly	Ile 1145		ту1	Phe	e Gly	Th:		er G	ly Th	nr G		la 155	Lys	Ser	Ala
Phe	Val 1160		: Phe	e Asr	ı Gly	Asr		nr T	yr Ty	yr Pl		sp 170	Ala .	Arg	Gly
His	Met 1175		. Thi	: Asr	n Ser	Glu 118	_	yr S	er Pi	ro As		ly 185	Lys .	Asp	Val
Tyr	Arg 1190		e Lev	ı Pro	) Asn	Gl <sub>y</sub>		le Me	et Le	∋u S∈		sn 200	Ala	Phe	Tyr
Ile	Asp 1205		a Asr	ı Gly	⁄ Asn	Th:		yr L	eu Ty	yr As		er 215	Lys ·	Gly	Gln
Met			Gly	gl <sub>y</sub>	y Tyr			ys Pl	ne As	∍p Va			Glu	Thr	Asp

											-00	.10 11	ruec		
	1220					1225					1230				
Lys	Asp 1235	_	. Lys	Glu	Ser	Lys 1240		Val	Lys	Phe	Arg 1245		Phe	Thr	
Asn	Glu 1250		Val	Met	Ala	Lys 1255		Val	Thr	Val	Ile 1260	Asp	Gly	Phe	
Thr	Gln 1265		Phe	Gly	Glu	Asp 1270		Phe	Gln	Ala	Lys 1275	Asp	ràs	Leu	
Val	Thr 1280		- Lys	Gly	Lys	Thr 1285		Tyr	Phe	Asp	Ala 1290	His	Thr	Gly	
Asn	Gly 1295		Lys	Asp	Thr	Trp 1300		Asn	Ile	Asn	Gly 1305	ГÀа	Trp	Tyr	
Tyr	Phe 1310		Ala	Asn	Gly	Val 1315		Ala	Thr	Gly	Ala 1320	Gln	Val	Ile	
Asn	Gly 1325		Lys	Leu	Tyr	Phe 1330		Glu	Asp	Gly	Ser 1335	Gln	Val	ГÀа	
Gly	Gly 1340		Val	Lys	Asn	Ala 1345	_	Gly	Thr	Tyr	Ser 1350	_	Tyr	ГÀа	
Glu	Gly 1355		Gly	Glu	Leu	Val 1360		Asn	Glu	Phe	Phe 1365	Thr	Thr	Asp	
Gly	Asn 1370		Trp	Tyr	Tyr	Ala 1375		Ala	Asn	Gly	Lys 1380	Thr	Val	Thr	
Gly	Ala 1385		Val	Ile	Asn	Gly 1390		His	Leu	Tyr	Phe 1395	Asn	Ala	Asp	
Gly	Ser 1400		Val	Lys	Gly	Gly 1405		Val	Lys	Asn	Ala 1410	Asp	Gly	Thr	
Tyr	Ser 1415		Tyr	Asn	Ala	Ser 1420		Gly	Glu	Arg	Leu 1425	Thr	Asn	Glu	
Phe	Phe 1430		Thr	Gly	Asp	Asn 1435		Trp	Tyr	Tyr	Ile 1440	Gly	Ala	Asn	
Gly	Lys 1445		Val	Thr	Gly	Glu 1450		Lys	Ile	Gly	Asp 1455	Asp	Thr	Tyr	
Phe	Phe 1460		Lys	Asp	Gly	Lys 1465		Val	Lys	Gly	Gln 1470	Thr	Val	Ser	
Ala	Gly 1475		Gly	Arg	Ile	Ser 1480		Tyr	Tyr	Gly	Asp 1485	Ser	Gly	ГÀз	
_	Ala 1490		Ser			Ile 1495					Gly 1500		Tyr	Val	
Tyr	Phe 1505		Lys	Asn	Gly	Leu 1510		Tyr	Pro	Pro	Arg 1515	Val	Leu	Asn	
<211 <212	0> SE L> LE 2> TY 3> OR	NGTH PE:	: 15 PRT	28	ptoc	occus	sal	ivar.	ius						
	)> SE				_										
			Lys		Thr	Gly L	ys I	le I		et G	lu As	n Ly:	s Val	l His	
Tyr	Lys		His 20	Lys	Val	Lys L	ys G 2		rp V	al T	hr Il	e Ala 30	a Val	l Ala	
Ser		Ala 35	Leu	Ala	Thr		al G O	ly G	ly L	eu S	er Al	a Thi	r Thi	r Ser	

Ser Val Ser Ala Asp Glu Thr Gln Asp Lys Ile Val Thr Gln Pro Asn 50 55 60

Leu 65	Asp	Thr	Thr	Ala	Asp 70	Leu	Val	Thr	Ser	Thr 75	Glu	Ala	Thr	Lys	Glu 80
Val	Asp	Lys	Arg	Thr 85	Asn	Thr	Lys	Glu	Ala 90	Asp	Val	Leu	Thr	Pro 95	Ala
Lys	Glu	Thr	Asn 100	Ala	Val	Glu	Thr	Ala 105	Thr	Thr	Thr	Asn	Thr 110	Gln	Ala
Thr	Ala	Glu 115	Ala	Ala	Thr	Thr	Ala 120	Thr	Thr	Ser	Asp	Val 125	Ala	Val	Ala
Ala	Val 130	Pro	Asn	Lys	Glu	Ala 135	Val	Val	Thr	Thr	Asp 140	Ala	Pro	Ala	Val
Thr 145	Thr	Glu	Lys	Ala	Glu 150	Glu	Gln	Pro	Ala	Thr 155	Val	Lys	Ala	Glu	Val 160
Val	Asn	Thr	Glu	Val 165	Lys	Ala	Pro	Gln	Ala 170	Ala	Leu	ГÀа	Asp	Ser 175	Glu
Val	Glu	Ala	Ala 180	Leu	Ser	Leu	Lys	Asn 185	Ile	Lys	Tyr	Thr	Asp 190	Gly	Lys
Tyr	Tyr	Tyr 195	Val	Asn	Glu	Asp	Gly 200	Ser	His	Lys	Glu	Asn 205	Phe	Ala	Ile
Thr	Val 210	Asn	Gly	Gln	Leu	Leu 215	Tyr	Phe	Gly	Lys	Asp 220	Gly	Ala	Leu	Thr
Ser 225	Ser	Ser	Thr	His	Ser 230	Phe	Thr	Pro	Gly	Thr 235	Thr	Asn	Ile	Val	Asp 240
Gly	Phe	Ser	Ile	Asn 245	Asn	Arg	Ala	Tyr	Asp 250	Ser	Ser	Glu	Ala	Ser 255	Phe
Glu	Leu	Ile	Asn 260	Gly	Tyr	Leu	Thr	Ala 265	Asp	Ser	Trp	Tyr	Arg 270	Pro	Val
Ser	Ile	Ile 275	Lys	Asp	Gly	Val	Thr 280	Trp	Gln	Ala	Ser	Thr 285	Ala	Glu	Asp
Phe	Arg 290	Pro	Leu	Leu	Met	Ala 295	Trp	Trp	Pro	Asn	Val 300	Asp	Thr	Gln	Val
Asn 305	Tyr	Leu	Asn	Tyr	Met 310	Ser	Lys	Val	Phe	Asn 315	Leu	Glu	Ala	Lys	Tyr 320
Thr	Ser	Thr	Asp	Lys 325	Gln	Ala	Asp	Leu	Asn 330	Arg	Ala	Ala	Lys	Asp 335	Ile
Gln	Val	Lys	Ile 340	Glu	Gln	Lys	Ile	Gln 345	Ala	Glu	Lys	Ser	Thr 350	Gln	Trp
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Lys	Glu 370	Thr	Glu	Asn	Tyr	Ser 375	Lys	Gly	Gly	Gly	Glu 380	Asp	His	Leu	Gln
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Leu	Ser	Val 35	Thr	Thr	Ser		/al 10	Ser	Ala	. Asr	G]	u Th: 45	r Glı	n Asp	. Lys
Thr	Val 50	Thr	Gln	Ser		Ser (	∃ly	Thr	Thr	Ala	8 Se		u Vai	l Thi	Ser
Pro 65	Glu	Ala	Thr	Lys	Glu 2 70	Ala <i>P</i>	/ap	Lys	Arg	Th: 75	: As	n Th	r Ly:	s Glu	ı Ala 80
Asp	Val	Leu	Thr	Pro 85	Ala :	Lys (	3lu	Thr	Asn 90	Ala	a Vá	al Gl	u Th:	r Ala 95	a Thr
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Thr	Asp	Ala	Pro	Ala	Val '	Thr T	Chr	Glu	Lys	Ala	a Gl	u Gl	u Glı	n Pro	) Ala

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Asn	Tyr 210	Leu	Asn	Tyr		Ser 215	Lys	Val	Phe	Asn	Le:	_	Ala	Lys	Tyr
Thr	Ser	Thr	Asp	Lys	Gln '	Val	Asp	Leu	Asn	Arg	Ala	a Ala	. Lys	a Asp	) Ile

Gln         Val         Lys         Ile         Gln         Lys         Ile         Gln         Ala         Lys         Far         Gln         Cln         Ala         Ile         Ser         Ala         Phe         Val         Lys         Thr         Gln         Pro         Gln         Trp         Asn           Lys         Glu         Thr         Glu         Asn         Phe         Ser         Lys         Gly         Gly         Glu         Asp         His         Leu         Gln           Gly         Ala         Leu         Leu         Asp         Asp         Pro         Arg         Thr         Ala         Asp         Pro         Arg         Thr         Ala         Asp         Pro         Arg         Thr         Ala         Asp         Pro         Arg         His         Leu         Asp         Asp         Asp         Pro         Arg         Thr         Ala         Asp         Pro         Arg
Lys Glu Thr Glu Asn Phe Ser Lys Gly Gly Gly Gly Gly Thr Asn Asn Asn Asn Asn Asn Thr Asn Asn Asn Asn Asn Asn Asn Asn Thr Asn Glu Thr Asn Asn Asn Asn Asn Asn Asn Asn Thr Asn Glu Thr Asn
Secondary   Seco
Ser       Asn       Tyr       Arg       Leu       Leu       Asn       Arg       Thr       Ala       Thr       Asn       Glu       Thr       Ala       Thr       Asn       Glu       Thr       Ala       Thr       Asn       Glu       Thr       Asn       Asn       Asn       Glu       Thr       Asn       A
310
325
Val         Gln         Ala glu         Gln         Leu         Asn gln         Ile His Tyr         Leu Met Ja65         Asn Trp         Gly Gly Gly Ja65           Ser         Ile Val         Met Gly         Asp Lys Asp Ja75         Asp Ala Asn Phe Ja80         Gly Ile Arg Val Arg Ja80         Val Asp Ja75         Asp Asp Asn Ja80         Gly Ile Arg Val Ja80         Val Asp Ja80         Met Leu Gln Leu Tyr Thr Asn Ja90         Asn Ja90         Asn Ja90         Val Asp Ja80         Ser Glu Ala Asn Ja80         Asn Ja90         Asn Ja90         Val Asp Ja90         Ser Glu Ala Asn Ja90         Asn Ja90         Asn Ja90         Val Asp Ja90         Asn Ja90
Ser       11e       Val       Met       Gly       Asp       Lys       Asp       Ala       Asn       Phe       Asp       Gly       Ile       Arg       Val         Asp       Ala       Val       Asp       Ala       Asp       Ala       Asp       Ala       Asp       Ala       Leu       Tyr       Asp       Asp       Met       Leu       Tyr       Tyr       Asp       Ala       Asp       Met       Leu       Glu       Tyr       Asp       Asp       Ala       Asp       Met       Ala       Asp       Ala       Leu       Ala       Asp       Asp       Ala       Asp       Asp       Ala       A
Asp Ala Val       Asp Asp Asn Sago       Asp Sago       Met Sago       Glu Glu Glu Gu Tyr Tyr Ago       Asp Asn Lys Sago       Asp Asn Asp Asn Ala Leu Ala Leu Ala Sago       Asp Asp Asn Ala Leu Ala Leu Ala Sago       Asp Asp Asn His Tyr Ala Sago       Asp Asp Asp Asn His Tyr Ala Sago       Asp Asp Asp Asp Asp Ala Sago       Asp Asp Asp Asp Ala Sago       Asp Ala Leu Ala Sago       Asp Asp Asp Asp Asp Ala Sago       Ala Clu Ala Sago       Arg Asp Asp Ala Sagoo       Arg Asp Asp Ala Sagoo       Ala Clu Ala Sagoo       Arg Asp Asp Ala Sagoo       Ala Sagoo       Arg Asp Asp Asp Ala Sagoo       Ala Sagoo       Ala Sagoo       Arg Asp Asp Asp Asp Sagoo       Ala Sago
385       390       395       400         Tyr Phe Arg Slu Tyr 405       Tyr Gly Val Asn Lys Ser Glu Ala Asn Ala Leu         Ala His Ile Ser Val Leu Glu Ala Trp Ser Leu Asn Asn Asn Asn His Tyr 430         Asn Asp Lys Thr Asp Gly Ala Ala Leu Ala Met Glu Asn Lys Gln Arg 435         Leu Ala Leu Leu Phe Ser Leu Ala Lys Pro Ile Lys Glu Arg Thr Pro 455         Ala Val Ser Pro Leu Tyr Asn Asn Asn Asn Thr Phe Asn Thr Thr Gln Arg 485         Glu Lys Thr Asp Trp 485       The Asn Lys Asp 490         Ala Clu Lys Thr Asp Trp 485       Thr Lys Asn Glu Lys Tyr Asn Glu Lys Tyr Asn Glu Lys Tyr Asp Glu Asp
Ala His Ile Ser Val Leu Glu Ala Trp Ser Leu Asn Asp Asn His Tyr 420   Asn Asp Lys Trr Asp Gly Ala Ala Leu Ala Met Glu Asn Lys Gln Arg 440   Leu Ala Leu Leu Phe Ser Leu Ala Lys Pro Ile Lys Glu Arg Thr Pro 460   Ala Val Ser Pro Leu Tyr Asn Asn Asn Thr Phe Asn Thr Thr Gln Arg Asp 480   Glu Lys Thr Asp Trp Ile Asn Lys Asp Asp Gly Ser Lys Ala Tyr Asn Glu Asp Glu Asp Gly Thr Val Lys Gln Ser Thr Ile Gly Lys Tyr Asn Glu Lys Tyr Asp Gly Thr Soo Ser Ser Ser Lys Ash Glu Lys Tyr Asn Glu Asp Asp Gly Gly Clys Tyr Ash Clys Tyr
Asn Asp Lys Thr Asp Gly Ala Ala Leu Ala Met Glu Asn Lys Gln Arg 455         Leu Ala Leu Leu Phe Ser Leu Ala Lys Pro Hor Asn Asn Lys Gln Arg 455         Ala Val Ser Pro Leu Tyr Asn Asn Lys Asn Lys Blu Lys Blu Lys Blu Lys Blu Arg Asn Asn Asn Blu Lys Blu Lys Blu Lys Blu Blu Lys Blu
Leu Ala Val Ser Pro Leu Ala Val Val Val Val Val Val Val Val Val V
Ala Val Ser Pro Leu Tyr Asn Asn Thr Phe Asn Thr Thr Gln Arg Asp 480  Glu Lys Thr Asp Trp 485
465 470 475 480  Glu Lys Thr Asp Trp Ile Asn Lys Asp Gly Ser Lys Ala Tyr Asn Glu 495  Asp Gly Thr Val Lys Gln Ser Thr 11e Gly Lys Tyr Asn Glu Lys Tyr 500 500 500 500 500 500 500 500 500 50
485 490 495  Asp Gly Thr Val Lys Gln Ser Thr Ile Gly Lys Tyr Asn Glu Lys Tyr 500 505 510
500 505 510
Gly Asp Ala Ser Gly Asn Tyr Val Phe Ile Arg Ala His Asp Asn Asn
515 520 525
Val Gln Asp Ile Ile Ala Glu Ile Ile Lys Lys Glu Ile Asn Pro Lys 530 535 540
Ser Asp Gly Phe Thr Ile Thr Asp Ala Glu Met Lys Lys Ala Phe Glu 545 550 550 560
Ile Tyr Asn Lys Asp Met Leu Ser Ser Asp Lys Lys Tyr Thr Leu Asn 565 570 575
Asn Ile Pro Ala Ala Tyr Ala Val Met Leu Gln Asn Met Glu Thr Ile 580 585 590
Thr Arg Val Tyr Tyr Gly Asp Leu Tyr Thr Asp Asp Gly His Tyr Met 595 600 605
Glu Thr Lys Ser Pro Tyr Tyr Asp Thr Ile Val Asn Leu Met Lys Asn 610 615 620
Arg Ile Lys Tyr Val Ser Gly Gly Gln Ala Gln Arg Ser Tyr Trp Leu 625 630 630 635 640
Pro Thr Asp Gly Lys Met Asp Lys Ser Asp Val Glu Leu Tyr Arg Thr 645 650 655

Asn	Glu	Val	Tyr 660	Thr	Ser	Val	Arg	Tyr 665	Gly	Lys	Asp	Ile	Met 670	Thr	Ala
Asp	Asp	Thr 675	Gln	Gly	Ser	Lys	Tyr 680	Ser	Arg	Thr	Ser	Gly 685	Gln	Val	Thr
Leu	Val 690	Val	Asn	Asn	Pro	Lys 695	Leu	Ser	Leu	Asp	Lys 700	Ser	Ala	Lys	Leu
Asp 705	Val	Glu	Met		Lys 710	Ile	His	Ala	Asn	Gln 715	Lys	Tyr	Arg	Ala	Leu 720
Ile	Val	Gly	Thr	Pro 725	Asn	Gly	Ile	Lys	Asn 730	Phe	Thr	Ser	Asp	Ala 735	Glu
Ala	Ile	Ala	Ala 740	Gly	Tyr	Val	Lys	Glu 745	Thr	Asp	Gly	Asn	Gly 750	Val	Leu
Thr	Phe	Gly 755	Ala	Asn	Asp	Ile	Lys 760	Gly	Tyr	Glu	Thr	Phe 765	Asp	Met	Ser
Gly	Phe 770	Val	Ala	Val		Val 775	Pro	Val	Gly	Ala	Ser 780	Asp	Asp	Gln	Aap
Ile 785	Arg	Val	Ala	Ala	Ser 790	Thr	Ala	Ala	Lys	Lys 795	Glu	Gly	Glu	Leu	Thr 800
Leu	Lys	Ala	Thr	Glu 805	Ala	Tyr	Asp	Ser	Gln 810	Leu	Ile	Tyr	Glu	Gly 815	Phe
Ser	Asn	Phe	Gln 820	Thr	Ile	Pro	Asp	Gly 825	Ser	Asp	Pro	Ser	Val 830	Tyr	Thr
Asn	Arg	Lys 835	Ile	Ala	Glu	Asn	Val 840	Asp	Leu	Phe	Lys	Ser 845	Trp	Gly	Val
Thr	Ser 850	Phe	Glu	Met	Ala	Pro 855	Gln	Phe	Val	Ser	Ala 860	Asp	Asp	Gly	Thr
Phe 865	Leu	Asp	Ser	Val	Ile 870	Gln	Asn	Gly	Tyr	Ala 875	Phe	Ala	Asp	Arg	Tyr 880
Asp	Leu	Ala	Met	Ser 885	ràa	Asn	Asn	Lys	Tyr 890	Gly	Ser	Lys	Glu	Asp 895	Leu
Arg	Asn	Ala	Leu 900	Lys	Ala	Leu	His	Lys 905	Ala	Gly	Ile	Gln	Ala 910	Ile	Ala
Asp	Trp	Val 915	Pro	Asp	Gln	Ile	Tyr 920	Gln	Leu	Pro	Gly	Lуs 925	Glu	Val	Val
Thr	Ala 930	Thr	Arg	Thr	Asp	Gly 935	Ala	Gly	Arg	Lys	Ile 940	Ser	Asp	Ala	Ile
Ile 945	Asp	His	Ser	Leu	Tyr 950	Val	Ala	Asn	Ser	Lys 955	Ser	Ser	Gly	Lys	Asp 960
Tyr	Gln	Ala	Lys	Tyr 965	Gly	Gly	Glu	Phe	Leu 970	Ala	Glu	Leu	ГЛа	Ala 975	Lys
Tyr	Pro	Glu	Met 980	Phe	ГÀв	Val	Asn	Met 985	Ile	Ser	Thr	Gly	Lys 990	Pro	Ile
Asp	Asp	Ser 995	Val	Lys	Leu	Lys	Gln 1000	_	Lys	s Ala	a Glu	1 Ty1		ne As	n Gly
Thr	Asn 1010		. Lev	ı Asp	Arg	101 101		al Gl	Ly Ty	r Va		eu S 020	Ser A	ap C	lu
Ala	Thr 1025	_	/ Lys	з Туг	Phe	Th:		al Th	ır Ly	/s G]		Ly <i>I</i> 035	Asn E	he 1	le
Pro	Leu 1040		ı Lev	ı Lys	Gly	Ası 104		lu Lχ	∕s Va	al II		nr (	Sly E	he S	er
Ser	Asp 1055	-	/ Lys	s Gly	⁄ Il∈	Th:	_	r Pł	ne Gl	Ly Th		er (	Gly A	Asn C	ln

Δla	Ive	Ser	Δla	Phe	Val	Thr	Phe	Agn	Glv	Agn	Thr	Tur	Tur	Phe
III	1070	501	1114	1110	V41	1075	1110	11011	O <sub>1</sub>		1080		- 7 -	1110
Asp	Ala 1085	Arg	Gly	His	Met	Val 1090	Thr	Asn	Gly	Glu	Tyr 1095	Ser	Pro	Asn
Gly	Lys 1100	Asp	Val	Tyr	Arg	Phe 1105	Leu	Pro	Asn	Gly	Ile 1110	Met	Leu	Ser
Asn	Ala 1115	Phe	Tyr	Val	Asp	Gly 1120	Asn	Gly	Asn	Thr	Tyr 1125	Leu	Tyr	Asn
Ser	Lys 1130	Gly	Gln	Met	Tyr	Lys 1135	Gly	Gly	Tyr	Ser	Lys 1140	Phe	Asp	Val
Thr	Glu 1145	Thr	Lys	Asp	Gly	Lys 1150		Ser	Lys	Val	Val 1155	Lys	Phe	Arg
Tyr	Phe 1160	Thr	Asn	Glu	Gly	Val 1165	Met	Ala	Lys	Gly	Val 1170		Val	Val
Asp	Gly 1175	Phe	Thr	Gln	Tyr	Phe 1180	Asn	Glu	Asp	Gly	Ile 1185	Gln	Ser	Lys
Asp	Glu 1190	Leu	Val	Thr	Tyr	Asn 1195	Gly	Lys	Thr	Tyr	Tyr 1200	Phe	Glu	Ala
His	Thr 1205	Gly	Asn	Ala	Ile	Lys 1210	Asn	Thr	Trp	Arg	Asn 1215		ГÀв	Gly
Lys	Trp 1220	Tyr	His	Phe	Asp	Ala 1225	Asn	Gly	Val	Ala	Ala 1230		Gly	Ala
Gln	Val 1235	Ile	Asn	Gly	Gln	His 1240	Leu	Tyr	Phe	Asn	Glu 1245	Asp	Gly	Ser
Gln	Val 1250	Lys	Gly	Gly	Val	Val 1255		Asn	Ala	Asp	Gly 1260		Phe	Ser
Lys	Tyr 1265	Lys	Asp	Gly	Ser	Gly 1270	Asp	Leu	Val	Val	Asn 1275	Glu	Phe	Phe
Thr	Thr 1280	Gly	Asp	Asn	Val	Trp 1285		Tyr	Ala	Gly	Ala 1290	Asn	Gly	Lys
Thr	Val 1295	Thr	Gly	Ala	Gln	Val 1300	Ile	Asn	Gly	Gln	His 1305	Leu	Phe	Phe
ГÀа	Glu 1310	Asp	Gly	Ser	Gln	Val 1315	Lys	Gly	Asp	Phe	Val 1320		Asn	Ser
Asp	Gly 1325	Thr	Tyr	Ser	Lys	Tyr 1330	Asp	Ala	Ala	Ser	Gly 1335	Glu	Arg	Leu
Thr	Asn 1340	Glu	Phe	Phe	Thr	Thr 1345	Gly	Asp	Asn	His	Trp 1350	Tyr	Tyr	Ile
Gly	Ala 1355	Asn	Gly	Lys	Thr	Val 1360	Thr	Gly	Glu	Val	Lys 1365	Ile	Gly	Asp
Asp	Thr 1370	Tyr	Phe	Phe	Ala	Lys 1375	Asp	Gly	Lys	Gln	Leu 1380	Lys	Gly	Gln
Ile	Val 1385	Thr	Thr	Arg	Ser	Gly 1390	Arg	Ile	Ser	Tyr	Tyr 1395	Phe	Gly	Asp
Ser	Gly 1400	Lys	Lys	Ala	Ile	Ser 1405	Thr	Trp	Val	Glu	Ile 1410	Gln	Pro	Gly
Val	Phe 1415	Val	Phe	Phe	Asp	Lys 1420	Asn	Gly	Leu	Ala	Tyr 1425	Pro	Pro	Glu
Asn	Met 1430	Asn												

<sup>&</sup>lt;210> SEQ ID NO 64 <211> LENGTH: 1532 <212> TYPE: PRT

365

-212 -	ODCANT CM.	Unknown	

<220> FEATURE:

<400> SEQUENCE: 64

Met Glu Asn Lys Val His Tyr Lys Leu His Lys Val Lys Lys Gln Trp

1 10 15

Val Thr Ile Ala Val Ala Ser Ala Ala Leu Ala Thr Val Val Gly Gly

Leu Ser Ala Thr Thr Ser Ser Val Ser Ala Asp Glu Thr Gln Asp Lys

Thr Val Thr Gln Pro Asn Ser Asp Thr Thr Ala Asp Leu Val Thr Ser 50 60

Thr Glu Ala Thr Lys Glu Val Asp Lys Arg Thr Asn Thr Lys Glu Ala 65 70 75 80

Asp Val Leu Thr Pro Ala Lys Glu Thr Asn Thr Val Glu Thr Ala Ala 85 90 95

Thr Thr Asn Thr Gln Ala Thr Ala Glu Ala Ala Lys Thr Ala Thr Thr

Thr Asn Thr Gln Ala Thr Ala Glu Val Ala Lys Thr Ala Thr Thr Ala 115 120 125

Asp Val Ala Val Ala Ala Val Pro Asn Lys Glu Ala Val Val Thr Thr 130 140

Asp Ala Pro Ala Val Thr Thr Glu Lys Ala Glu Glu Gln Pro Ala Thr 145 150 155 160

Val Lys Ala Glu Val Val Asn Thr Glu Val Lys Ala Pro Glu Ala Ala 165 170 175

Leu Lys Asp Ser Glu Val Glu Ala Ala Leu Ser Leu Lys Asn Ile Lys 180 185 190

Asn Ile Asp Gly Lys Tyr Tyr Tyr Val Asn Glu Asp Gly Ser His Lys 195 200 205

Glu Asn Phe Ala Ile Thr Val Asn Gly Gln Leu Leu Tyr Phe Gly Lys 210 215 220

Asp Gly Ala Leu Thr Ser Ser Ser Thr Tyr Ser Phe Thr Gln Gly Thr 225 230 235 240

Thr Asn Ile Val Asp Gly Phe Ser Ile Asn Asn Arg Ala Tyr Asp Ser 245 250 255

Ser Glu Ala Ser Phe Glu Leu Ile Asp Gly Tyr Leu Thr Ala Asp Ser 260 265 270

Trp Tyr Arg Pro Ala Ser Ile Ile Lys Asp Gly Val Thr Trp Gln Ala 275 280 285

Ser Thr Ala Glu Asp Phe Arg Pro Leu Leu Met Ala Trp Trp Pro Asn 290 295 300

Val Asp Thr Gln Val Asn Tyr Leu Asn Tyr Met Ser Lys Val Phe Asn 305 310 315 320

Leu Asp Ala Lys Tyr Ser Ser Thr Asp Lys Gln Glu Thr Leu Lys Val \$325\$ 330 335

Ala Ala Lys Asp Ile Gln Ile Lys Ile Glu Gln Lys Ile Gln Ala Glu  $340 \hspace{1.5cm} 345 \hspace{1.5cm} 350 \hspace{1.5cm}$ 

Lys Ser Thr Gln Trp Leu Arg Glu Thr Ile Ser Ala Phe Val Lys Thr

Gln Pro Gln Trp Asn Lys Glu Thr Glu Asn Tyr Ser Lys Gly Gly 370 \$375\$

Glu Asp His Leu Gln Gly Gly Ala Leu Leu Tyr Val Asn Asp Ser Arg

<sup>&</sup>lt;223> OTHER INFORMATION: unknown Streptococcus species

205															
385					390					395					400
Thr	Pro	Trp	Ala	Asn 405	Ser	Asn	Tyr	Arg	Leu 410	Leu	Asn	Arg	Thr	Ala 415	Thr
Asn	Gln	Thr	Gly 420	Thr	Ile	Asp	Lys	Ser 425	Ile	Leu	Asp	Glu	Gln 430	Ser	Asp
Pro	Asn	His 435	Met	Gly	Gly	Phe	Asp 440	Phe	Leu	Leu	Ala	Asn 445	Asp	Val	Asp
Leu	Ser 450	Asn	Pro	Val	Val	Gln 455	Ala	Glu	Gln	Leu	Asn 460	Gln	Ile	His	Tyr
Leu 465	Met	Asn	Trp	Gly	Ser 470	Ile	Val	Met	Gly	Asp 475	Lys	Asp	Ala	Asn	Phe 480
Asp	Gly	Ile	Arg	Val 485	Asp	Ala	Val	Asp	Asn 490	Val	Asp	Ala	Asp	Met 495	Leu
Gln	Leu	Tyr	Thr 500	Asn	Tyr	Phe	Arg	Glu 505	Tyr	Tyr	Gly	Val	Asn 510	Lys	Ser
Glu	Ala	Asn 515	Ala	Leu	Ala	His	Ile 520	Ser	Val	Leu	Glu	Ala 525	Trp	Ser	Leu
Asn	Asp 530	Asn	His	Tyr	Asn	Asp 535	Lys	Thr	Asp	Val	Ala 540	Ala	Leu	Ala	Met
Glu 545	Asn	Lys	Gln	Arg	Leu 550	Ala	Leu	Leu	Phe	Ser 555	Leu	Ala	Lys	Pro	Ile 560
Lys	Glu	Arg	Thr	Pro 565	Ala	Val	Ser	Pro	Leu 570	Tyr	Asn	Asn	Thr	Phe 575	Asn
Thr	Thr	Gln	Arg 580	Asp	Glu	Lys	Thr	Asp 585	Trp	Ile	Asn	ГÀа	Asp 590	Gly	Ser
Lys	Ala	Tyr 595	Asn	Glu	Asp	Gly	Thr 600	Val	Lys	Lys	Ser	Thr 605	Ile	Gly	Lys
Tyr	Asn 610	Glu	Lys	Tyr	Gly	Asp 615	Ala	Ser	Gly	Asn	Tyr 620	Val	Phe	Ile	Arg
Ala 625	His	Asp	Asn	Asn	Val 630	Gln	Asp	Ile	Ile	Ala 635	Glu	Ile	Ile	Lys	Lys 640
Glu	Ile	Asn	Glu	Lys 645	Ser	Asp	Gly	Phe	Thr 650	Ile	Thr	Asp	Ser	Glu 655	Met
Lys	Arg	Ala	Phe 660	Glu	Ile	Tyr	Asn	Lys 665	Asp	Met	Leu	Ser	Asn 670	Asp	Lys
Lys	Tyr	Thr 675	Leu	Asn	Asn	Ile	Pro 680	Ala	Ala	Tyr	Ala	Val 685	Met	Leu	Gln
Asn	Met 690	Glu	Thr	Ile	Thr	Arg 695	Val	Tyr	Tyr	Gly	Asp 700	Leu	Tyr	Thr	Asp
Asp 705	Gly	Asn	Tyr	Met	Glu 710	Ala	Lys	Ser	Pro	Tyr 715	Tyr	Asp	Thr	Ile	Val 720
Asn	Leu	Met	Lys	Ser 725	Arg	Ile	Lys	Tyr	Val 730	Ser	Gly	Gly	Gln	Ala 735	Gln
Arg	Ser	Tyr	Trp 740	Leu	Pro	Thr	Asp	Gly 745	Lys	Met	Asp	ГÀв	Ser 750	Asp	Val
Glu	Leu	Tyr 755	Arg	Thr	Asn	Glu	Val 760	Tyr	Thr	Ser	Val	Arg 765	Tyr	Gly	Lys
Asp	Ile 770	Met	Thr	Ala	Asp	Asp 775	Thr	Gln	Gly	Ser	Lys 780	Tyr	Ser	Arg	Thr
Ser 785	Gly	Gln	Val	Thr	Leu 790	Val	Val	Asn	Asn	Pro 795	Lys	Leu	Thr	Leu	Asp 008
Gln	Ser	Ala	Lys	Leu 805	Asn	Val	Val	Met	Gly 810	Lys	Ile	His	Ala	Asn 815	Gln

ГЛа	Tyr	Arg	Ala 820	Leu	Ile	Val	Gly	Thr 825	Pro	Asn	Gly	Ile	830		. Phe
Thr	Ser	Asp 835	Ala	Glu	Ala	Ile	Ala 840	Ala	Gly	Tyr	Val	Lys 845		Thr	Asp
Gly	Asn 850	Gly	Val	Leu	Thr	Phe 855	Gly	Ala	Asn	Asp	Ile 860		Gly	Tyr	Glu
Thr 865	Phe	Asp	Met	Ser	Gly 870	Phe	Val	Ala	Val	Trp 875	Val	Pro	Val	Gly	Ala 880
Ser	Asp	Asp	Gln	Asp 885	Ile	Arg	Val	Ala	Ala 890	Ser	Thr	Ala	Ala	Lys 895	Lys
Glu	Gly	Glu	Leu 900	Thr	Leu	Lys	Ala	Thr 905	Glu	Ala	Tyr	Asp	Ser 910		Leu
Ile	Tyr	Glu 915	Gly	Phe	Ser	Asn	Phe 920	Gln	Thr	Ile	Pro	Asp 925		Ser	Asp
Pro	Ser 930	Val	Tyr	Thr	Asn	Arg 935	Lys	Ile	Ala	Glu	Asn 940	Val	Asp	Leu	Phe
Lys 945	Ser	Trp	Gly	Val	Thr 950	Ser	Phe	Glu	Met	Ala 955	Pro	Gln	Phe	Val	Ser 960
Ala	Asp	Asp	Gly	Thr 965	Phe	Leu	Asp	Ser	Val 970	Ile	Gln	Asn	Gly	Tyr 975	Ala
Phe	Ala	Asp	Arg 980	Tyr	Asp	Leu	Ala	Met 985	Ser	Lys	Asn	Asn	990		Gly
Ser	Lys	Glu 995	Asp	Leu	Arg	Asn	Ala 1000		ı Ly:	s Ala	a Le		s L 05	ys A	la Gly
Ile	Gln 1010		a Ile	e Ala	a Asp	Tr <sub>1</sub>		al Pi	ro A	sp G		le 020	Tyr	Gln	Leu
Pro	Gly 1025		Glu	ı Val	. Val	. Thi 103		la Ti	nr A	rg Tl		ap 035	Gly	Ala	Gly
Arg	Lys 1040		e Ser	: Asp	) Ala	116 104		le As	sp H	is S		eu 050	Tyr	Val	Ala
Asn	Ser 1055		s Sei	Ser	Gly	Ly:		ap Ty	yr G	ln A		ys 065	Tyr	Gly	Gly
Glu	Phe 1070		ı Ala	a Glu	ı Leu	Ly:		la Ly	ys T	yr P:		lu 080	Met	Phe	ГÀа
Val	Asn 1089		∶Il∈	e Ser	Thr	109		ys Pi	ro I	le A		ap 095	Ser	Val	ГÀа
Leu	Lys 1100		n Trp	Lys	a Ala	110		yr Pl	ne A	sn G		hr 110	Asn	Val	Leu
Asp	Arg 1115		/ Val	. Gly	y Tyr	112		eu Se	er A	ap G		la 125	Thr	Gly	ГÀа
Tyr	Phe 1130		r Val	Thr	: Lys	Gl: 113		ly As	sn Pl	ne I		ro 140	Leu	Gln	Leu
ГÀа	Gly 1145		ı Lys	s Lys	Val	. Ile 115		hr G	ly Pl	ne Se		er 155	Asp	Gly	ГЛа
Gly	Ile 1160		туг	Ph∈	e Gly	Th:		er G	ly A	sn G		la 170	Lys	Ser	Ala
Phe	Val 1175		. Phe	Asn	ı Gly	Ası 118		hr Ty	yr T	yr Pl		sp 185	Ala	Arg	Gly
His	Met 1190		l Thi	: Asn	ı Gly	Gli 119		yr Se	er P:	ro A:		ly 200	Lys	Asp	Val
Tyr	Arg	Phe	e Leu	ı Pro	) Asn	Gly		le Me	et L	eu Se		sn 215	Ala	Phe	Tyr

Val	Asp 1220	_	Asn	Gly	Asn	Thr 1225		Leu	Tyr	Asn	Ser 1230	Lys	Gly	Gln				
Met	Tyr 1235	Lys	Gly	Gly	Tyr	Ser 1240		Phe	Asp	Val	Thr 1245	Glu	Thr	ГЛа				
Asp	Gly 1250	_	Glu	Ser	Lys	Val 1255	Val	Lys	Phe	Arg	Tyr 1260	Phe	Thr	Asn				
Glu	Gly 1265	Val	Met	Ala	Lys	Gly 1270	Val	Thr	Val	Val	Asp 1275	Gly	Phe	Thr				
Gln	Tyr 1280		Asn	Glu	Asp	Gly 1285		Gln	Ser	Lys	Asp 1290	Glu	Leu	Val				
Thr	Tyr 1295		Gly	Lys	Thr	Tyr 1300		Phe	Glu	Ala	His 1305	Thr	Gly	Asn				
Ala	Ile 1310		Asn	Thr	Trp	Arg 1315	Asn	Ile	Lys	Gly	Lys 1320	Trp	Tyr	His				
Phe	Asp 1325	Ala	Asn	Gly	Val	Ala 1330	Ala	Thr	Gly	Ala	Gln 1335	Val	Ile	Asn				
Gly	Gln 1340	His	Leu	Tyr	Phe	Asn 1345		Asp	Gly	Ser	Gln 1350	Val	ГЛа	Gly				
Ser	Ile 1355		ГЛа	Asn	Ala	Asp 1360		Thr	Phe		Lys 1365	Tyr	ГЛа	Asp				
Ser	Ser 1370	Gly	Asp	Leu	Val	Val 1375	Asn	Glu	Phe	Phe	Thr 1380	Thr	Gly	Asp				
Asn	Val 1385	Trp	Tyr	Tyr	Ala	Gly 1390	Ala	Asn	Gly	Lys	Thr 1395	Val	Thr	Gly				
Ala	Gln 1400	Val	Ile	Asn	Gly	Gln 1405	His	Leu	Phe	Phe	Lys 1410	Glu	Asp	Gly				
Ser	Gln 1415		Lys	Gly	Asp	Phe 1420		Lys	Asn		Asp 1425	Gly	Thr	Tyr				
Ser	Lys 1430	Tyr	Asp	Ala	Ala	Ser 1435	Gly	Glu	Arg	Leu	Thr 1440	Asn	Glu	Phe				
Phe	Thr 1445	Thr	Gly	Asp	Asn	His 1450	Trp	Tyr	Tyr	Ile	Gly 1455	Ala	Asn	Gly				
Lys	Thr 1460	Val	Thr	Gly	Glu	Val 1465	Lys	Ile	Gly	Asp	Asp 1470	Thr	Tyr	Phe				
Phe	Ala 1475		Asp	Gly	Lys	Gln 1480		Lys	Gly	Gln	Ile 1485	Val	Thr	Thr				
Arg	Ser 1490		Arg	Ile	Ser	Tyr 1495	Tyr	Phe	Gly	Asp	Ser 1500	Gly	Lys	Lys				
Ala	Ile 1505	Ser	Thr	Trp	Val	Glu 1510	Ile	Gln	Pro	Gly	Val 1515	Phe	Val	Phe				
Phe	Asp 1520	-	Asn	Gly	Leu	Ala 1525	_	Pro	Pro	Glu	Asn 1530	Met	Asn					

## What is claimed is:

- 1. A reaction solution comprising water, sucrose and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 93% identical to SEQ ID NO:10.
- 2. The reaction solution of claim 1, wherein said glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having at least 50% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100.
- 3. The reaction solution of claim 2, wherein said glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having

- 55 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100.
- 4. The reaction solution of claim 3, wherein said glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 250.
  - 5. The reaction solution of claim 1, further comprising a primer.
- ${\bf 6}.$  The reaction solution of claim 5, wherein the primer is  $_{65}$  dextran.
  - 7. The reaction solution of claim 5, wherein the primer is hydrolyzed glucan.

- **8**. The reaction solution of claim **1**, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 95% identical to SEQ ID NO:10.
- **9**. The reaction solution of claim **8**, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 97% identical to SEO ID NO:10.
- 10. The reaction solution of claim 9, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 99% identical to SEQ ID NO:10.
- 11. The reaction solution of claim 1, wherein a heterologous amino acid sequence of 1-300 residues is at the N-terminus and/or C-terminus of said glucosyltransferase enzyme.
- 12. A method for producing poly alpha-1,3-glucan comprising:
  - a) contacting at least water, sucrose, and a glucosyltransferase enzyme that synthesizes poly alpha-1,3-glucan, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 93% identical to SEQ ID NO:10;

whereby poly alpha-1,3-glucan is produced; and

- b) optionally, isolating the poly alpha-1,3-glucan produced in step (a).
- 13. The method of claim 12, wherein said glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having at  $_{25}$  least 50% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100.

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- 14. The method of claim 13, wherein said glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 100.
- 15. The method of claim 14, wherein said glucosyltransferase enzyme synthesizes poly alpha-1,3-glucan having 100% alpha-1,3 glycosidic linkages and a number average degree of polymerization of at least 250.
- 16. The method of claim 12, wherein step (a) further comprises contacting a primer with the water, sucrose, and glucosyltransferase enzyme.
  - 17. The method of claim 16, wherein the primer is dextran.
- 18. The method of claim 16, wherein the primer is hydrolyzed glucan.
- 19. The method of claim 12, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 95% identical to SEQ ID NO:10.
- **20**. The method of claim **19**, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 97% identical to SEQ ID NO:10.
- **21**. The method of claim **20**, wherein said glucosyltransferase enzyme consists of an amino acid sequence that is at least 99% identical to SEQ ID NO:10.
- 22. The method of claim 12, wherein a heterologous amino acid sequence of 1-300 residues is at the N-terminus and/or C-terminus of said glucosyltransferase enzyme.

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